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# THE HARVEY SOCIETY

# THE HARVEY LECTURES

Delivered under the auspices of

THE HARVEY SOCIETY OF NEW YORK

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# THE HARVEY LECTURES

DELIVERED UNDER THE AUSPICES OF

## THE HARVEY SOCIETY OF NEW YORK

UNDER THE PATRONAGE OF THE NEW YORK ACADEMY OF MEDICINE

1924-1925

BY

DR. S. P. L. SÖRENSEN
DR. WILLIAM SNOW MILLER
DR. A. V. HILL
DR. THORVALD MADSEN
DR. WILLEM EINTHOVEN
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## SERIES XX

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# PREFACE

THE present volume of Harvey Society Lectures, the twentieth in the series, offers to the medical public eight lectures by investigators, each eminent in his field. The subject matter covers a wide range. It includes the fruit of years of personal research by the authors and brings up to date the subjects discussed. To these lecturers, American and foreign, who have so generously contributed to this series, the Harvey Society expresses its obligation and its thanks. For permission to publish the lecture of Dr. S. P. L. Sörensen, the Editor wishes to thank the American Chemical Society.

GEORGE M. MACKENZIE, *Secretary.*

March, 1926.

# THE HARVEY SOCIETY

A SOCIETY FOR THE DIFFUSION OF KNOWLEDGE OF THE MEDICAL SCIENCES

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### I.

This Society shall be named the Harvey Society.

### II.

The object of this Society shall be the diffusion of scientific knowledge in selected chapters in anatomy, physiology, pathology, bacteriology, pharmacology, and physiological and pathological chemistry, through the medium of public lectures by men who are workers in the subjects presented.

### III.

The members of the Society shall constitute three classes: Active, Associate, and Honorary members. Active members shall be laboratory workers in the medical or biological sciences, residing in the City of New York, who have personally contributed to the advancement of these sciences. Associate members shall be meritorious physicians who are in sympathy with the objects of the Society, residing in the City of New York. Members who leave New York to reside elsewhere may retain their membership. Honorary members shall be those who have delivered lectures before the society and who are neither active nor associate members. Associate and honorary members shall not be eligible to office, nor shall they be entitled to a vote.

Members shall be elected by ballot. They shall be nominated to the Executive Committee and the names of the nominees shall accompany the notice of the meeting at which the vote for their election will be taken.

IV.

The management of the Society shall be vested in an executive committee to consist of a President, a Vice-President, a Secretary, a Treasurer, and three other members, these officers to be elected by ballot at each annual meeting of the Society to serve one year.

V.

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# * THE SOLUBILITY OF PROTEINS

DR. S. P. L. SÖRENSEN

Director of the Carlsberg Laboratories, Copenhagen, Denmark

ONE of the most important questions today concerning protein studies is how far it is possible to apply to protein solutions the theories derived from the study of real solutions; or whether such colloid solutions as those of protein are so divergent in their behavior from ordinary real solutions that to parallel the behavior of colloid solutions and real solutions must be considered inadvisable, or even warned against, as Wo. Ostwald has often done.

I believe there is a growing tendency to think that Ostwald is not right in his view of the matter, but that the need simply is, by improved methods and particularly by the development of more exact analytical procedures to make it possible to deal with protein solutions in accordance with general physicochemical views.

The space allowed does not admit of a more particular discussion of the great work already done in this direction; I must be content to mention a few names in order to throw some light on the nature of the researches here under discussion.

The investigations of Harriette Chick and C. W. Martin [1] on the coagulation of protein have demonstrated, among other things, that the denaturation process is a monomolecular process, provided that the hydrogen-ion concentration be kept constant.

The elaborate investigations of Wolfgang Pauli and his collaborators on proteins, especially in acid or alkaline solutions, should also be mentioned. Pauli has succeeded in showing that proteins form salts of the same nature and character as simple ampholytes.

Further should be mentioned Jacques Loeb, recently and prematurely deceased, whose studies on the so-called "Donnan equilibrium" in connection with the physicochemical properties

* Lecture delivered October 4, 1924.

of protein solutions have thrown light on so many hitherto unknown matters.

Finally, among all these examples, to which many more might be added, I may perhaps venture to mention the investigations on hen's egg albumin conducted in the Carlsberg Laboratory, by which we succeeded in demonstrating that in many and essential cases the theories developed from the study of real solutions may be applied also to the solutions of egg albumin. This is true, for example, in such important cases as the state of equilibrium by salt precipitation, the application of Gibbs' phase rule, the question of the osmotic pressure of albumin solutions and in many more instances.

The present discussion, then, concerns the precipitation of some proteins, and the conditions of their solubility in water and salt solutions, these conditions being but imperfectly known, yet of no small importance.

I shall first briefly mention the equilibrium conditions of albumin when crystallized by means of ammonium sulfate and then somewhat more fully discuss the solubility of the globulins of horse serum, more particularly that of euglobulin.

### THE PRECIPITATION OF ALBUMINS BY AMMONIUM SULFATE

If Gibbs' phase rule, which applies to real solutions, be applicable also to protein solutions, a precipitation of the solution by means of a salt must result in the same state of equilibrium between the precipitate and the surrounding mother liquor, irrespective of whether the initial solution be concentrated or diluted, if only care be taken that, at the end of the process, the factors that influence the precipitation (temperature, salt concentration, hydrogen-ion concentration) are alike in all cases. Under such conditions much albumin should be precipitated from a strong albumin solution; and from a weak solution, on the other hand, only little; but the final albumin concentration in the solution must in every case be the same, provided that Gibbs' phase rule applies.

However, according to the generally accepted view, the pro-

tein solution does not behave in accordance with the phase rule, the common opinion being that, under otherwise equal circumstances, the protein is salted out the more completely, the higher the starting protein concentration. It is not necessary to enter on a discussion of the voluminous literature published on this subject. We may be content to elucidate the question by a single example. For this purpose I choose Harriette Chick and C. W. Martin's [2] otherwise most valuable work on the precipitation of egg albumin by ammonium sulfate, because the conditions of the experiments mentioned there are analogous to those under which

TABLE 1

*Precipitation of Pure Egg-albumin with Ammonium Sulfate. Influence of Concentration of Protein*

Ratio salt/water = 31/100. Concentration of protein varying

| Albumin G. | Water G. | Salt G. | G. of albumin in 100 g. of total mixture | G. of albumin to 31 g. of salt and 100 g. of $H_2O$ | G. of albumin in 100 g. of filtrate | G. of albumin precipitated from 100 g. of total mixture | Protein pptd. % |
|---|---|---|---|---|---|---|---|
| 1.90 | 56.92 | 17.65 | 2.481 | 3.33 | 1.130 | 1.351 | 54.4 |
| 1.90 | 36.92 | 11.44 | 3.775 | 5.14 | 1.115 | 2.660 | 70.4 |
| 3.79 | 50.85 | 15.76 | 5.383 | 7.45 | 1.159 | 4.224 | 78.5 |
| 7.59 | 53.69 | 16.64 | 9.738 | 14.13 | 0.935 | 8.803 | 90.4 |
| 4.74 | 17.31 | 5.36 | 17.306 | 27.4 | 0.772 | 16.534 | 95.5 |

our experiments were conducted. In a series of experiments, reproduced from their paper in Table 1, Chick and Martin investigated the influence of the initial albumin concentration on the precipitation of egg albumin by ammonium sulfate. In all the experiments of the series, the ratio, *ammonium sulfate to water, was constant, namely, 31: 100, but the amount of albumin* varied in the mixtures from 3.3 to 27.4 g. per 100 g. of water. As may be seen from the sixth vertical column in the table, Chick and Martin find that the albumin is precipitated more completely, the higher the initial concentration.

Now, the error is due to the fact that Chick and Martin have taken it for granted that the proportion between ammonium sulfate and water does not change during the precipitation, and

consequently they have made no determination of the amount of ammonium sulfate in the filtrates. It is, however, beyond doubt that a solution of egg albumin behaves during precipitation just as during crystallization, in which latter case we have demonstrated that 1 g. of egg albumin contains constantly 0.22 g. of water. If, however, it is clearly realized that the egg albumin by its precipitation removes water from the solution, it is evident that the higher the starting concentration of egg albumin is, the more water will be removed from the solution during the precipitation, and consequently the concentration of ammonium sulfate in the filtrate will be the higher. As an increase of the ammonium sulfate concentration causes a more complete precipitation of the egg albumin, Chick and Martin's experimental results may, therefore, be accounted for.

Indeed, the experiments made by us in order to throw light on this question have proved that the amount of albumin remaining in the mother liquor by the crystallization of egg albumin is independent of the initial protein concentration, if only care be taken that at the end of the crystallization the ammonium sulfate concentration of the mother liquor is the same in all the experiments and that this is really so, must, of course, be demonstrated analytically.

In figure 1 is shown graphically the result of one of our series of experiments, the content of egg albumin in the mother liquor being used as ordinate and the crystallization time in days as abscissa. In all the experiments the ammonium sulfate concentration is the same, namely, 26.658 g. of ammonium sulfate per 100 g. of water; but the initial albumin concentration varies in the different experiments from 2.3 to 14.1 g. of albumin per 100 g. of water. There is a curve corresponding to each experiment, and it is plainly shown in the diagram that when the crystallization has proceeded for a few days, the amount of albumin in all the filtrates is practically the same. A more minute consideration of the experimental results will show that the small discrepancies between the results point in the opposite direction to what would be expected according to the above-mentioned generally accepted

theory; and besides, it is easy to give an explanation of these small discrepancies.

From what I have here stated, as well as from a series of other experiments which also include serum albumin, but which would carry us too far to enter on now, it is apparent that the behavior of albumins when precipitated with ammonium sulfate does not exclude the application of the phase rule in its usual form and with its usual consequences. What has been stated here about the precipitation of albumins by ammonium sulfate is probably also true of many other protein precipitations and whenever the opposite view has hitherto been commonly held, the reason must be sought in the defective experimental treatment of the subject.

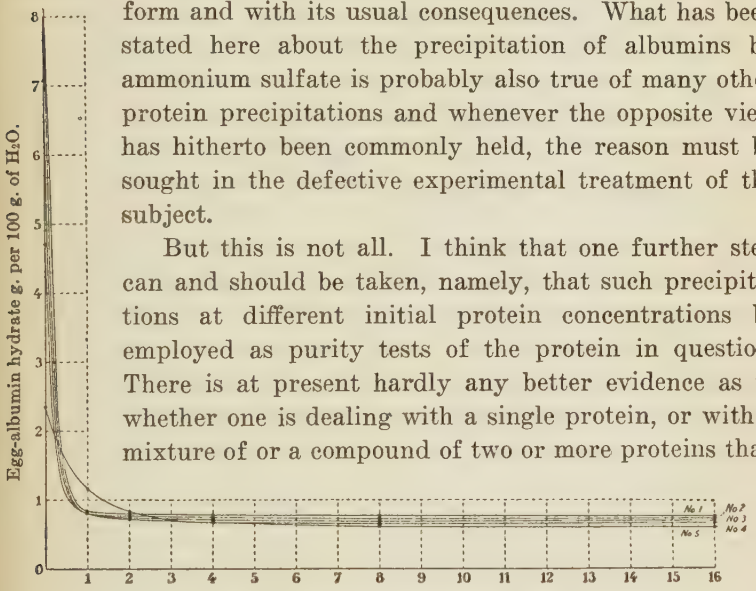


FIG. 1.—Time for crystallization. Days.

But this is not all. I think that one further step can and should be taken, namely, that such precipitations at different initial protein concentrations be employed as purity tests of the protein in question. There is at present hardly any better evidence as to whether one is dealing with a single protein, or with a mixture of or a compound of two or more proteins than just such precipitations at different starting concentrations of protein, and under such conditions that salt concentrations and hydrogen-ion concentration are alike in all the experiments after the precipitation.

For the further elucidation of this I shall refer only to one instance. We have tested the degree of purity of a sample of pseudoglobulin prepared from horse serum and very carefully purified by repeated fractionated precipitation, dialysis, solution in pure water and renewed fractionated precipitation. In figure 2 are graphically reproduced the results of five precipitation

experiments, in all of which the ammonium sulfate concentration was 24.533 g. per 100 g. of water and the starting concentration of globulin varied from 5.6 g. of hydrated globulin (Curve I) to 0.28 g. (Curve V) per 100 g. of water. As in figure 1, the precipitation time expressed in days is used as abscissa and the globulin content of the mother liquor, as ordinate. It will be seen from the diagram that the globulin concentration of the mother liquor is by no means lower, the higher the starting concentration. On the contrary, the globulin concentration in the mother liquor is higher, the *higher* the starting concentration; and there is no doubt that this pseudoglobulin sample, in spite of the careful purification and fractionation, is not a homogeneous substance but, as demonstrated by other experiments on which I shall hardly have time to touch, probably consists of a combination of several globulin complexes. Possibly the small discrepancies observed in the experiments with egg albumin (Fig. 1) may be explained in the same way.

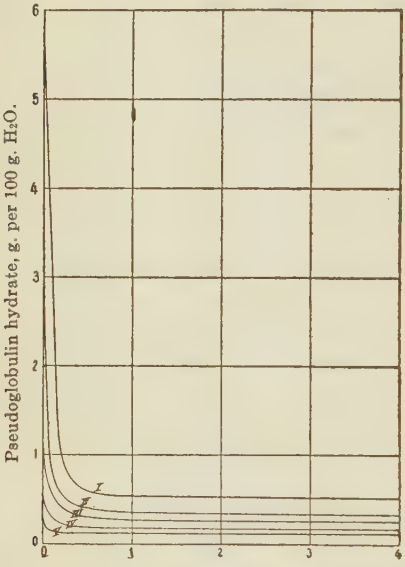


FIG. 2.—Time for precipitation. Days.

SOLUBILITY OF EUGLOBULIN IN WATER AND WEAK SALT SOLUTIONS

There are in serum two globulins: *euglobulin*, which contains phosphorus, is insoluble in water but soluble in weak salt solutions and is precipitated from its solutions by the addition of saturated ammonium sulfate solution to the extent of half the volume of the solution; and *pseudoglobulin*, which is free from phosphorus, is soluble in water and is precipitated completely

from its solutions by the addition of an equal volume of saturated ammonium sulfate solution.

I shall not go into details regarding the methods we have adopted for the separation of euglobulin and pseudoglobulin, but merely mention that neither fractionation nor dialysis, nor a combination of the two methods, results in a complete separation of the two globulins. I have already mentioned an instance which proved that even very carefully purified pseudoglobulin is not a single substance and in the following I shall mention a series of experiments which serve to show that it is not possible to prepare a pure euglobulin in this way either. In so doing I shall also have the opportunity of thoroughly elucidating the peculiar solubility conditions of euglobulin, which can be explained only from the viewpoint of a conception of the inter-relation of globulins which differs from the one hitherto accepted.

In all probability globulins occur in the serum itself as well as in the special globulin fractions obtained from it, not as mixtures of the two globulins, but as combinations of them. As to the nature of the combinations between the globulins, whether we have before us true compounds, as I am most inclined to think, or whether it is a question of solid solutions, or of a reciprocal adsorption between the different globulins, I shall venture no statement.

If a euglobulin complex or molecule be designated $E$, and a pseudoglobulin complex or molecule, $P$, and if, for a provisional first guidance, we start out on the basis of the (most probably erroneous) supposition that the respective eu- or pseudoglobulin complexes are all alike, then such a combination may be written $EpPq$. The combination is readily dissolved in water and dilute salt solutions as long as the pseudoglobulin is in great excess, but gradually as the pseudoglobulin is split off by a simple dissociation process—as by diluting the solution with water—the remaining combination becomes richer and richer in euglobulin and therefore still more sparingly soluble and at last precipitates. Very characteristic of such a dissociation process is the circumstance that we are here in the presence of a real reciprocal alter-

ation of equilibrium, so that the reaction (dilution with water, for example) does not occur *solely* between the combinations present in the solution, but the precipitate already once formed, takes part in the reaction until the new equilibrium is established between precipitate and mother liquor, corresponding to the decreased salt concentration brought about by the addition of water. Of course, the order of the process is reversed if the salt concentration be increased. I shall not go further into this, but merely mention that the behavior of salt precipitation in large salt concentrations may be explained from quite similar views.

The most elaborate researches on the solubility of euglobulin have been carried out by I. Mellanby [3] and W. B. Hardy.[4] In these classical works it is stated that the solubility of euglobulin in neutral salt solutions increases with the salt concentration where fairly dilute salt concentrations are in question, but it is also stated that the amount of globulin dissolved by any given salt concentration is approximately proportional to the total amount of globulin employed in the solubility experiment. This highly peculiar circumstance, which we have found corroborated by our experiments can be explained, according to our general chemical and physicochemical theories, only on the basis of the above-mentioned assumption that the euglobulin employed is not a single substance, but can be dissociated by salt solutions. I shall here give but a brief account of a few of our series of experiments.

To begin, I would mention that we have found also that the solubility of euglobulin increases with the increased salt concentration, at any rate as long as fairly small concentrations are in

TABLE 2

| | | | |
|---|---|---|---|
| Total globulin used, mg. *N* ..... | 29.06 | 58.11 | 116.22 |
| 100 cc. of sat. soln. contains, mg. *N* .... | 10.95 | 21.75 | 42.15 |

question. Furthermore, a globulin obtained by dialysis and subsequent treatment with water, after several washings with water

showed the solubility in 0.02 *N* sodium chloride solution given in Table 2.

TABLE 3

*Purification of the Globulin Fraction* $B_{III}$

| Nature of the globulin fraction | Solubility of the globulin fraction in 0.02 *N* NaCl-solution: Total amount of globulin nitrogen per 100 cc. used for the experiment mg. | Amount of globulin nitrogen dissolved per 100 cc. of solution mg. | Mg. of P per g. of globulin nitrogen |
|---|---|---|---|
| | 26.22 | 3.23 | |
| $B_{III}$ | 52.44 | 5.39 | 4 |
| | 78.66 | 6.89 | |
| $B_{IV}$ | 11.92 | 1.13 | |
| ($B_{III}$ dissolved in 125 cc. of $H_2O$ + 15 cc. of *N* NaCl, precipitated with 1 litre of $H_2O$; decantation of the mother liquor, washing with 250 cc. of $H_2O$, decantation of the washing water and suspension of $B_{IV}$ in 200 cc. of $H_2O$) | 23.84 | 1.64 | 4 |
| | 35.76 | 2.05 | |
| $B_V$ | 20.71 | 0.82 | |
| ($B_{IV}$ dissolved in 140 cc. of $H_2O$ + 30 cc. of *N* NaCl, precipitated with 1 litre of $H_2O$; decantation of the mother liquor, washing with 200 cc. of $H_2O$, decantation of the washing water and suspension of $B_V$ in 95 cc. of $H_2O$) | 41.42 | 0.87 | 6 |
| | 62.13 | 1.28 | |
| $B_{VI}$ | 17.08 | 0.62 | |
| ($B_V$ dissolved in 45 cc. of $H_2O$ + 35 cc. of *N* NaCl, precipitated with 850 cc. of $H_2O$; decantation of the mother liquor, washing with 190 cc. of $H_2O$, decantation of the washing water and suspension of $B_{VI}$ in 100 cc. of $H_2O$) | 34.16 | 0.62 | 10 |
| | 51.24 | 0.72 | |

The solubility of this well-washed sample of euglobulin is thus very nearly proportional to the amount of globulin employed in the solubility experiment, just as stated by Mellanby and Hardy.

A really effective purification cannot, however, be obtained

by washing with water, but only by dissolving the euglobulin in a weak salt solution and subsequent precipitation by dilution with water. To throw light on this question I shall mention a series of experiments, in which the purification of a globulin fraction, $B_{III}$, was followed in all its details. In the experiment, $B_{III}$, which was taken as the primary substance had already been reprecipitated once and was consequently rather sparingly soluble. It was further reprecipitated three times by solution in sodium chloride

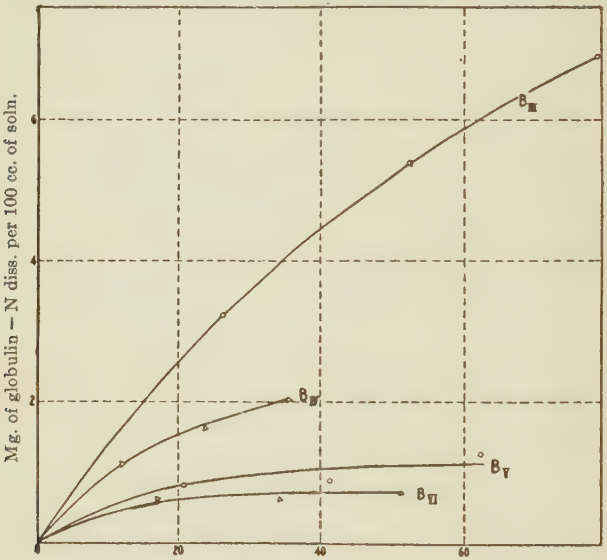


FIG. 3.—Total mg. of globulin – N per 100 cc.

solution and precipitation by water. $B_{III}$ itself, as well as the products, $B_{IV}$, $B_V$, $B_{VI}$, obtained by the reprecipitations, was subjected to an analysis by which the solubility in 0.02 *N* sodium chloride solution was determined, three different but known globulin amounts of each fraction being used in the solubility determinations.

The analytical results are grouped in Table 3 and graphically reproduced in figure 3, in which the total amount of globulin nitrogen is used as abscissa, while the globulin nitrogen in 100 cc. of saturated solution is used as ordinate.

From the table as well as from the figure it appears that the purification of the globulin not only causes a decrease of its solubility, but also makes it approximately constant and independent of the total amount of globulin employed in the solubility experiment; graphically, this is shown by the curves in figure 3 more and more approaching a parallel with the abscissa axis.

If it were really a question of a single substance, this substance should have a constant solubility in water or in salt solution of a given concentration, irrespective of whether the surplus of globulin left undissolved is large or small. As just mentioned, however, euglobulin does not behave in this manner, neither after thorough washing with water nor after several reprecipitations.

Euglobulin can be treated so long and so often with water that the pseudoglobulin complexes, which can be dissociated in pure water are really split off and the remaining substance will consequently show—at least approximately—a constant solubility in water, independent of the amount of globulin employed. Thus Edwin J. Cohn,[5] in a series of carefully conducted and recently published experiments, has found that the solubility in pure water of well-washed euglobulin corresponds to about 1.2 mg. of globulin nitrogen per 100 cc. of saturated solution. We have been able to obtain exactly similar results, but if the purification of the globulin was continued, or was conducted not only by washing with water, but also by reprecipitation by means of neutral salt solutions, the reprecipitated globulin exhibited a considerably decreased solubility in water; furthermore, as shown in Table 3, such an adequately reprecipitated fraction $B_{VI}$ exhibits an approximately constant solubility in 0.02 *N* sodium chloride solution, which is lower than the solubility in pure water stated by Cohn.

However, even a substance such as $B_{VI}$ cannot be regarded as a pure euglobulin; it exhibits, indeed, a fairly constant solubility in water and such weak sodium chloride solutions as 0.02 *N*, but in stronger salt solutions, where the dissociation of pseudo-

globulin complexes can be carried still further, such a globulin preparation as $B_{VI}$ shows a solubility dependent on the amount of globulin employed. On account of lack of material, we have unfortunately not been able to conduct such a solubility experiment with $B_{VI}$ but we have done so with another preparation called $\alpha_{a_4}$.

The globulin sample $\alpha_{a_4}$ was purified by means of a series of reprecipitations, and was consequently one of the most sparingly soluble globulin fractions we have had to deal with. With this sample of globulin a series of solubility experiments was conducted in rather strong potassium chloride solutions, and with three different quantities of globulin in each concentration of potassium chloride. The experimental results are grouped in Table 4 and graphically reproduced in figure 4, in which the potassium chloride concentration is used as abscissa, while the dissolved euglobulin in terms of milligrams of nitrogen per 100 cc. of saturated solution is used as ordinate.

TABLE 4

*Solubility of the Globulin Fraction $\alpha_{a_4}$ in Solutions of Potassium Chloride*

Equilibrium by precipitation; 1 day's standing; 18°

| Concentration of potassium chloride *N* | Total globulin nitrogen per 100 cc. | | |
|---|---|---|---|
| | Mg. | Mg. | Mg. |
| .. | 4.53 | 9.31 | 18.61 |
| | Globulin nitrogen dissolved per 100 cc. of soln. | | |
| 0.05 | 0.62 | 0.98 | 1.70 |
| .10 | 2.63 | 4.22 | 6.33 |
| .20 | 4.12 | 7.57 | 12.25 |
| .50 | 4.17 | 9.27 | 18.07 |
| 1.00 | 4.12 | 9.27 | 18.27 |
| 2.00 | 3.91 | 8.18 | 16.00 |
| 2.80 | .. | 5.20 | 7.36 |

From the table, as well as from the figure, it is apparent that the solubility of $\alpha_{a_4}$ in these comparatively strong potassium chloride solutions is largely dependent on the quantity of globulin employed in the solubility experiment, so that we here find exactly similar conditions to those indicated by solubility deter-

minations of less thoroughly purified euglobulin samples in water or dilute salt solutions.

It is further seen that in these experiments such salt concentrations have been used that the solubility of the globulin again decreases, no doubt owing to the precipitation of com-

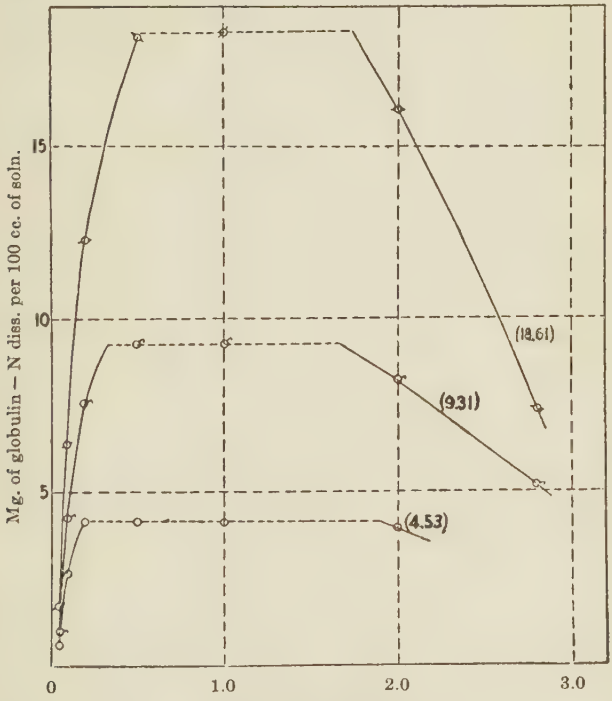


FIG. 4.—KCl – concn.

binations between the globulin and the salt or one of its components. The middle parts of the curves are dotted, because in these cases we have hardly had wholly saturated solutions; at any rate, in these experiments the residue was extremely slight and the presence of small quantities of denaturized globulin must always be reckoned with.

We have in these experiments a striking instance of the fact that even a highly purified euglobulin can be further split up by

dissolving it in concd. potassium chloride solution if the subsequent dilution with water is so slight that the precipitation of the globulin is only partial. When, on the other hand, the addition of water is abundant, all the globulin is precipitated anew. By further addition of water, the precipitate first deposited will combine with the globulin complexes present in the solution until a state of equilibrium corresponding to the decreased salt concentration has been established between the precipitate and the globulin in the solution. That such an interaction occurs between the precipitate first deposited and the globulin present in the solution, and that it is not a question of a continuous precipitation of the same compound, can be demonstrated by a fractionated precipitation with water. This fractionation must naturally be so conducted that the first deposit, which must contain relatively the least amount of pseudoglobulin and consequently be most sparingly soluble, is filtered off before a further addition of water takes place.

Such a fractionation was conducted with $\alpha_{a_4}$, which was dissolved in 0.5 *N* potassium chloride solution; from this solution $\alpha_{a_4}$I was precipitated by the addition of a little water, from the filtrate of this precipitate by addition of more water, $\alpha_{a_4}$II, and finally from the filtrate of this precipitate, $\alpha_{a_4}$III.

Of the solubility of these globulin fractions in 0.1 *N* potassium chloride solution as compared with the solubility of the original substance, $\alpha_{a_4}$, figure 5 gives particulars.

It appears from the figure, in which the total amount of globulin nitrogen is used as abscissa, while the dissolved amount of globulin nitrogen per 100 cc. of saturated solution is taken as ordinate, that the three fractions had widely different solubility and that—as was to be expected—especially Fraction I, but also very markedly Fraction II, showed less solubility; Fraction III, on the other hand, showed greater solubility than the starting material, $\alpha_{a_4}$.

The peculiar solubility conditions, characteristic for euglobulin, which are mentioned here, can scarcely be explained in any other way than that I have adduced, namely, on the basis

of the supposition that ordinary euglobulin must be regarded as a combination of euglobulin and pseudoglobulin, *EpPq*.

I should like to discuss the solubility conditions of pseudoglobulin as fully as I have discussed those of euglobulin, but time will not permit. I must be content to point out a single circumstance, which is closely connected with that which I have just stated about euglobulin.

Ordinary pseudoglobulin solution, in my opinion, is also composed of a complex such as *EpPq*, but with a very slight pro-

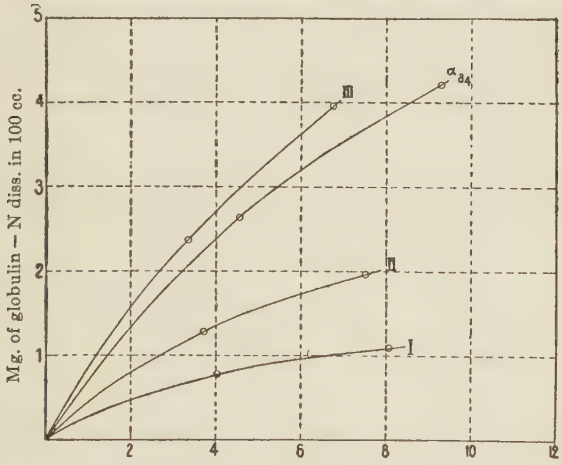


FIG. 5.—Total globulin – N mg.

portion of euglobulin to that of pseudoglobulin, as the quite minimal content of phosphorus goes to show. When such a solution is precipitated by ammonium sulfate solution, the precipitate first deposited, which is richest in euglobulin, will not remain passive by the further precipitation but will, when the state of equilibrium is altered by renewed addition of salt, combine with the pseudoglobulin of the solution. Therefore, the solution, being precipitated to the same ammonium sulfate concentration, will be poorer in globulin if the precipitate first deposited is allowed to be present during the continued precipitation than if it is filtered off before the precipitation is proceeded with.

In figure 6 the bottom curve shows such a precipitation of a pseudoglobulin solution to different ammonium sulfate concentrations which are used as abscissas, while the ordinates are the quantities of globulin left in the solution. The one above the bottom curve shows the same conditions for the same globulin solution, but such that the solution has first been precipitated

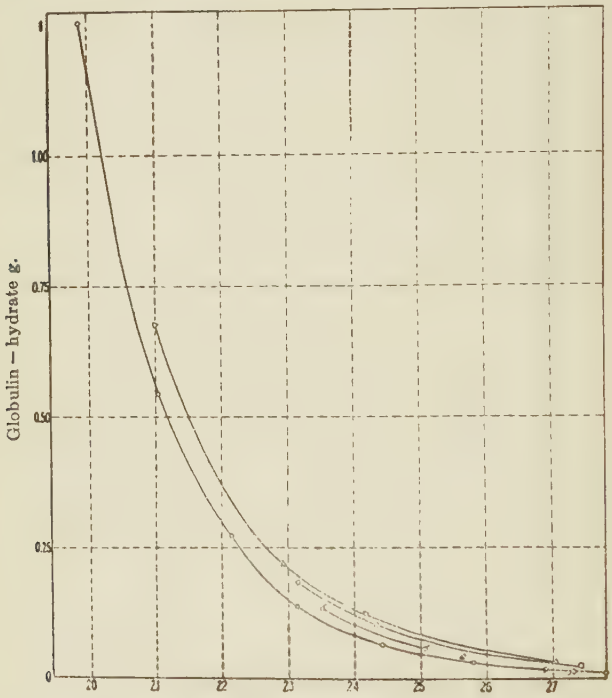


FIG. 6.—$(NH_4)_2SO_4$, g.

to an ammonium sulfate concentration of 20 g. per 100 g. of water, whereupon the precipitate has been filtered off, and the precipitation of the filtrate continued *without the presence of the first precipitate.* It is evident from the curves that, under otherwise equal conditions, the precipitate is greater and the amount of globulin in the filtrate is therefore less, when the first precipitate is allowed to remain.

I perfectly realize that some of the phenomena which I have adduced here come under what is generally termed "mechanical carrying down" or "adsorption"; others come under the so-called "protective colloid" category. The reason why I have left these categories out of consideration is that I have desired to make an attempt at approaching the question somewhat more closely than it is possible by the application of these very capacious, but not very elucidating classification terms.

Only by the application of purely chemical and physicochemical theories shall we succeed, I think, in bringing this very important problem of serum globulins, which occupies so many minds but is still unsolved, one step further toward solution.

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# STUDIES ON THE NORMAL AND PATHOLOGICAL HISTOLOGY OF THE LUNG[1]

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FROM various studies on the normal and pathological histology of the lung I have selected the ultimate arrangement of the air-spaces and the distribution of smooth muscle for my topics on the normal side, and the relation of reticulum to the tuberculous processes occurring in the human lung, on the pathological side.

It would be interesting to follow the gradual evolution of our present conception of the ultimate arrangement of the air-spaces did time permit. I can discuss only some of the more salient points.

In 1661, Malpighi showed that the conception of the lung being composed of "spumous blood or bloody spume," as described by Vesalius, was erroneous, and that the air and blood were contained in separate vessels. He also showed that the lung had a cellular structure.

Passing over the work of Willis, Reisseisen, von Sömmering, Moleschott, and other worthy investigators, we come to the year 1846, in which Rossignol described the lungs as being formed of successive bronchial ramifications which terminated by suddenly dilating into the form of funnels (*infundibula*), the internal walls of which were lined with alveoli. Alveoli also lined the last two or three divisions of the bronchial tubes. The introduction of the term *infundibulum* was unfortunate, for this misnomer has occasioned much confusion in interpreting the structure of the lung.

Rossignol defines an infundibulum as "a small sac, more or less conical in shape, having its internal surface partitioned off by numerous alveoli, and having but a single opening of com-

[1] Lecture delivered October 25, 1924.

munication with the external air'' (Fig. 1). Along the bronchial tubes his alveoli were formed by partitions projecting into their lumina and not, as is now known, by evaginations from their surface. His conception of there being only one communication between an infundibulum and the external air was correct; but his conception of the relation which the infundibula bore to the last division of the bronchial tree was incorrect.

In 1852 Kölliker published the first edition of his *Mikroskopische Anatomie.* The scheme which he there gave (Fig. 2) of the air-spaces connected with the last division of the bronchial tree is familiar to the older members of this Society, and is even now occasionally republished. In the preceding scheme of Rossignol the last division of the bronchial tree terminated in an infundibulum, which often showed a partial subdivision. In the scheme of Kölliker the last division of the bronchial tree terminated in a pyriform *sacculus,* which had opening into it a group of air-cells, or pulmonary vesicles. Because of this grouping of the vesicles, which he compared to the lobules of a racemous gland, he said that there was no occasion to designate them infundibula, as was done by Rossignol. On the other hand, the sacculi of Kölliker and the infundibula of Rossignol are identical and constitute the finest lobules (*primary lobules*) in contradistinction to the *secondary lobules,* which were composed of a variable number of primary lobules and were marked out by connective-tissue septa. The alveoli of the infundibulum of Rossignol always opened directly into it, while the alveoli of Kölliker did not always open directly into a sacculus, but opened first into other alveoli and through them into the common cavity of the sacculus.

In 1860 the Fothergillian Gold Medal was awarded to A. T. Houghton Waters for his essay on *The Anatomy of the Human Lung.* This is the most exhaustive and accurate piece of work which had been done on the lung up to that time and, had modern methods of reconstruction been available, he would, no doubt, have been able to solve the perplexing problem of the structure of the lung.

According to Rossignol and to Kölliker, the last division of

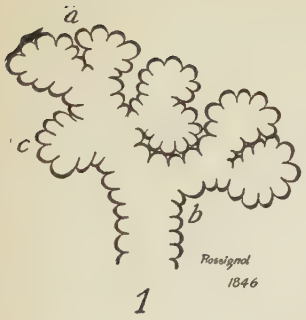
the bronchial tree ended in a single cavity, called by the former an *infundibulum,* by the latter a *sacculus.* Waters rejects all previous nomenclature and calls the last division of the bronchial tree a *terminal bronchial tube;* sometimes he uses as a synonym, *ultimate bronchial tube.* The large air-spaces connected with a terminal bronchial tube he calls *air-sacs*; the small projections from the surface of the terminal bronchial tubes and from the air-sacs he calls *alveoli,* a term first used by Rossignol. It is from his nomenclature that *terminal bronchus* and *air-sac* have passed into common usage in English and American descriptions of pulmonary structure.

While in the schemes of Rossignol and of Kölliker only a single air-sac was connected with the last division of the bronchial tree, Waters found that a group of air-sacs was connected with each terminal bronchial tube. These air-sacs did not communicate with each other; neither did the group of air-sacs that were connected with one terminal bronchial tube communicate with those of an adjoining group. Sometimes an air-sac would present a deep cleft on its more distal margin, indicating a partial subdivision (Fig. 3).

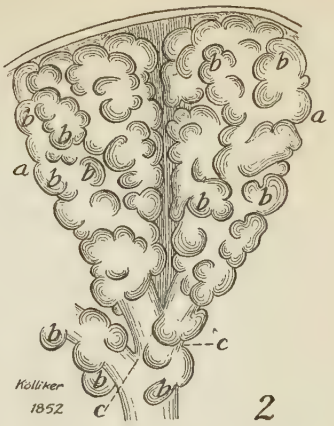
Waters introduced the term *lobulette* (little lobule) in the place of lobule, to designate the group of air-sacs connected with the terminal bronchial tube. This did not meet with favor; the term *lobule* has been retained and has its original significance, that is, *the last division of the bronchial tree and the air-spaces connected with it.*

In tracing the course of the air-sacs Waters found that, as they pass from the periphery of the lobule, they diminish in size and eventually open into the dilated end of a terminal bronchial tube. Sometimes two or three air-sacs joined together before opening into the dilated end of a terminal bronchial tube which he calls the *point de réunion* of all the air-sacs.

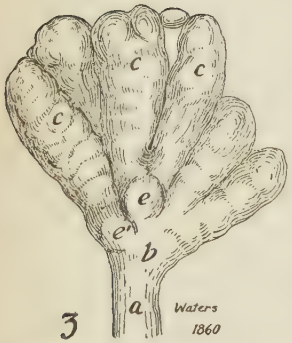
Alveoli were, according to Waters, present not only over the surface of the air-sacs, but also along the terminal bronchial tube. Rossignol called special attention to the alveoli in this situation. He found them along the last two or three divisions of the bronchial tree. Waters could only demonstrate them on his


a
c
b
Rossignol
1846

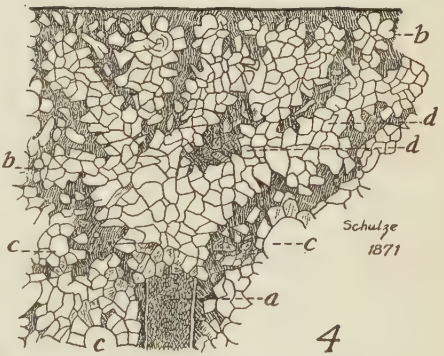
1


a
b
c
Kölliker
1852

2


c
e
e'
b
a
Waters
1860

3


b
d
d
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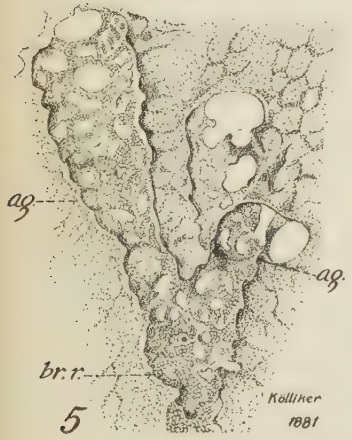
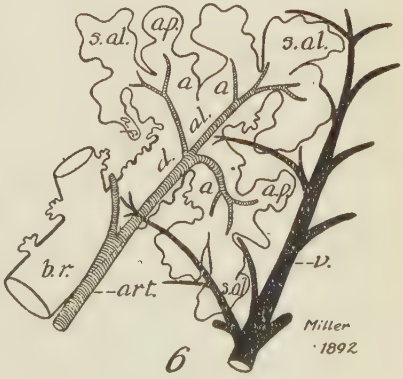
c
a
Schulze
1871

4


ag.
ag.
br. r.
Kölliker
1881

5


s.al.
ap.
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a
a
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ap.
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art.
s.al.
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Miller
1892

6

terminal bronchial tube, and thought it possible that Rossignol drew his conclusion from observations on the cat's lung in which, he says, they are more abundant than in the human lung. Rossignol was right in his statement, however, as reconstruction methods have demonstrated.

The diagram of F. E. Schulze (Fig. 4) is still used by many of the Continental writers to explain the normal and pathological histology of the lung. In order to obtain an intelligent understanding of this diagram it will be necessary to follow closely his description.

Each lung, he says, is supplied with a single bronchus which, as soon as it has penetrated into the substance of the lung, breaks up into a series of dichotomous branches which pursue a straight course until they reach a position near the surface of the lung. Each of these branches gives off laterally, in a spiral manner, still smaller branches, which also have a straight course. The lateral branches to which these smaller branches give origin break up into terminal branches which again have a straight course. By this mode of branching the smallest bronchi take a peculiar zigzag course. The smallest of the above series of branches (Fig. 4, *a*) terminate in the respiratory cavities. These cavities also consist of round tubes which break up, by a dichotomous method of division, into two to four terminal twigs which again have short lateral branches. The terminal twigs and their lateral branches end in a blind, funnel-shaped cavity, which Schulze calls an *infundibulum.*

The course of these various divisions of the original bronchus is indeed a zigzag one; but we eventually arrive at the same type of ending as in the earlier schemes (Waters excepted), namely, an infundibulum.

The cylindrical passages which are situated between the last of the bronchial subdivisions and the infundibula do not have smooth walls, but are lined with numerous alveoli. For this reason Schulze has named them *alveolar passages* (*Alveolengänge*).

For the first time we have a name for that subdivision of the bronchial tree which possesses alveoli and is situated between the

last bronchial tube with smooth walls and an infundibulum. This constitutes the contribution of Schulze to our knowledge of pulmonary architecture. In his study of the histological structure of the lung he made other and valuable contributions, some of which will be discussed in connection with my second topic.

In 1881 Kölliker again studied the structure of the lung, mainly in connection with its epithelium. We have seen that Schulze gave the name *alveolar passage* to the long cylindrical tube, beset on all sides with alveoli and situated between a smooth bronchial tube and an infundibulum. Kölliker goes a step further, and divides this long alveolar passage (*Alveolargang*) into two parts, depending on the type of the epithelium. That part which is situated more deeply in the substance of the lung he named *bronchiolus respiratorius* (respiratory bronchiole), because it took part in respiration; while for that part which was directly connected with an infundibulum he retained the name introduced by Schulze, *Alveolargang* (*alveolar passage*). Both of these subdivisions possess alveoli, but they are more abundant along the alveolar passage.

Before proceeding further, we may bring together the various points which the investigators under consideration have worked out. Rossignol showed that after various subdivisions the bronchial tubes terminated in a funnel-shaped dilatation which he called an *infundibulum,* and that alveoli were present along the last two or three divisions of the bronchial tree. Kölliker did not add, at this time, anything of importance to the architecture of the lung. He confirmed in nearly every point the work of Rossignol. He did add to our knowledge of the histological structure of various parts of the lungs; but this is foreign to our subject. Waters made a very material addition to our knowledge in showing that a number of air-sacs (infundibula) were connected with each terminal bronchial tube. Schulze ignored the work of Waters, and returned to the idea of a single infundibulum forming the ultimate ending of each bronchial tube. He gave a name (*Alveolargang*) to the divisions of the bronchial tree first described by Rossignol as possessing alveoli. Finally, Kölliker subdivided the *Alveolargang* of Schulze into two parts,

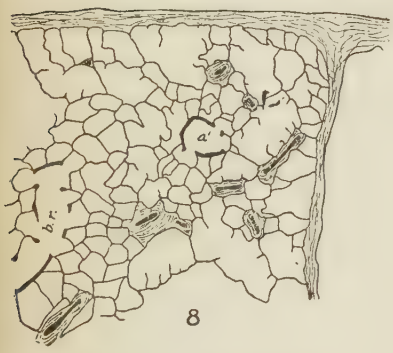
*bronchiolus respiratorius* and *Alveolargang,* and this latter name was changed to *ductulus alveolaris* when the *B. N. A.* nomenclature came into use (1895).

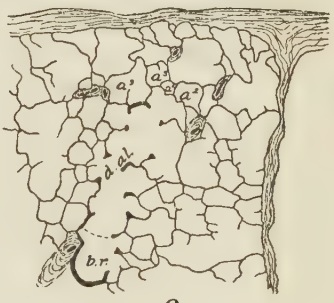
We now have the following nomenclature for the last divisions of the bronchial tree: *bronchiolus; bronchiolus respiratorius; Alveolargang;* and a group of elongated, more or less funnel-shaped *infundibula* or *air-sacs,* attached to its distal end. It remains to be seen whether these groups of elongated infundibula cannot be further subdivided.

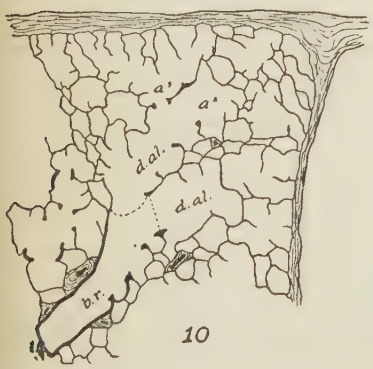
In the early part of 1887 I spent some time working in the Pathological Laboratory of the College of Physicians and Surgeons, New York. At that time Professor Delafield was supporting, in the face of much opposition, the thesis that there existed intercommunication between the air-vesicles. The opportunity came to me, shortly after leaving this laboratory, to take up the study of the structure of the lung, and it has been my life-work since that time. At first, my results apparently confirmed Delafield's statement that the "air-passages seem to be made up of a succession of large vesicles opening into each other." J. West Roosevelt came to his assistance, and showed that if a complete corrosion of the lung be picked to pieces, under a dissecting microscope, small more or less spheroidal bodies could be isolated, and that these bodies had two or more facets which represented the points at which they communicated with similar bodies. I repeated Roosevelt's method and obtained the same results. For the time being it appeard that Delafield's statement was correct, but reconstruction methods gave very different results.

As one studies the diagrams and descriptions of the older investigators, it seems as though they were constantly looking for a single section which was cut through as many subdivisions of an air-passage and its ending as possible (Fig. 7). Only one investigator (Waters) could visualize more than one air-sac (we will drop the term *infundibulum*) as being connected with a terminal division of the bronchial tree.

When the diagram of Waters is carefully studied there can be recognized, in its centre, a place where three air-sacs join



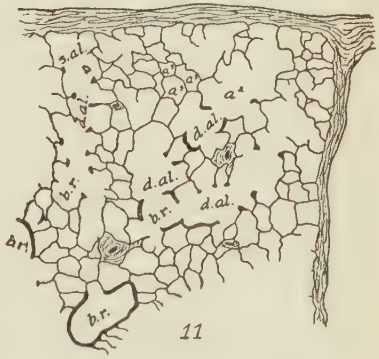




FIG. 7.—Camera lucida tracing from a section of a human lung in which a bronchiolus, two bronchioli respiratorii, a ductulus alveolaris, an atrium, and a sacculus alveolaris with its alveoli, are cut longitudinally. This section was taken 6 cm. below the pleura and formed part of a secondary lobule. The connective-tissue septa between the adjoining lobules forms the boundary of the section.

FIGS. 8–11.—For full description of these figures see the text. Reference lettering is the same for all four figures. *b. r.*, bronchiolus respiratorius; *a, a, a:* portions of three atria; *s. al.*: sacculus alveolaris. The sections are bounded above by the pleura and on the right by a septum which separates two adjoining secondary lobules from each other.

before entering the dilated extremity of the terminal bronchial tube (Fig. 3, *c, c, e*) ; two of them are represented in their full length while one of them is represented as being cut off. A somewhat similar condition can be seen at their right where two air-sacs join.

Serial sections and reconstruction methods had not been used, or thought of, at that time; and Waters failed to realize that this union of air-sacs and the accompanying external markings suggested a special cavity interposed between the air-sacs and terminal bronchial tubes. As soon as reconstruction methods were applied to the study of the architecture of the lung this cavity became an actuality.

If a reconstruction be made of the air-spaces connected with the last division of the bronchial tree, it matters not whether it be from a cat, dog or man, if this reconstruction be cut along a line which continues the long axis of the *ductulus alveolaris* the diagram shown in figure 6 will be obtained.

In this diagram a *bronchiolus respiratorius,* of Kölliker, is seen to divide into two *ductuli alveolares* (*Alveolargänge*) of Schulze. Only one *ductulus* is carried out in full detail. Opening out from this latter by nearly circular openings are three *atria* (Fig. 6, *a, a, a*). Each atrium is interposed between a group of *sacculi alveolares* (air-sacs) and the expanded end of the *ductulus alveolaris,* while small evaginations of the walls of the *sacculi* form the *alveoli pulmonis.* Alveoli are also present along the *bronchiolus respiratorius,* the *ductuli alveolaris* and the *atria.* The elongated air-sacs of Waters have now been divided into two parts, namely, *atrium* and *air-sacs.*

If the diagram of Waters be again studied, the opening leading out from the expanded end of his terminal bronchial tube, which he describes as opening into an air-sac (Fig. 3, *e*) may be interpreted as leading into an atrium and not into an air-sac. It was isolated atria that both Roosevelt and myself obtained when wax corrosions of the lung were picked apart. They are the only portions of a corrosion of the normal air-spaces that show more than one facet.

In the preceding schemes of the ultimate arrangement of the

air-spaces within the lung, the lobule, no matter how constituted, has a more or less triangular outline; and the bronchial tube, no matter how named, enters at its apex, with the air-sacs continuing in the general direction of the bronchial tube.

In figure 6 the same triangular outline appears as in the previous figures, but the *ductulus alveolaris* does not join the apex of the triangle: it enters at one side. This point of entrance and the presence of atria allow a greater range of direction to the air-sacs, and some of them, instead of continuing the direction of the *ductulus* toward the periphery of the lobule, have a course in the opposite direction and come to be situated close to the *ductulus* with which they are connected. Because of their direction they may be called *recurrent air-sacs* and they often form the apex of the triangle.

In order that the relation of the various air-spaces shown in figure 6 may be better understood, and to demonstrate more clearly the relations of atria, photomicrographs of sections which entered into a reconstruction of the air-spaces of the human lung were made and reproduced as line drawings.

In figure 8, $a^1$, an opening is seen marked out by heavy outlines. This leads into an atrium that is situated above the level of the section (compare Fig. 3, *e*). Following the air-spaces toward the lower left corner of the section, a chain of small air-spaces (alveoli) can be recognized. These are sections of alveoli which open into the underlying *ductulus alveolaris* and *bronchiolus respiratorius.* The heavy outlines on the left side of the section mark out the walls of a *bronchiolus respiratorius* that belongs to another system of air-spaces.

Figure 9 is a section taken 160 micra below figure 8, and shows not only the *ductulus* with which atrium $a^1$ communicates, but also a portion of the *bronchiolus respiratorius* from which the *ductulus arises.* Only a reconstruction from serial sections will bring out a differentiation of this nature. Around the distal end of the *ductulus* four air-spaces ($a^2$, $a^2$, $a^3$, $a^3$) are indicated. These belong to two atria, with corresponding numbers, which are connected with the *ductulus* at a lower level.

Figure 10 is a section taken 240 micra below figure 9, and

shows the *bronchiolus respiratorius* cut lengthwise and the two *ductuli alveolares* into which it divides. The openings by which atrium $a^2$ and atrium $a^3$ communicate with the *ductulus* appear in this section. Two air-sacs (*sacculi alveolares*) open out of atrium $a^3$, but none of the air-sacs belonging to atrium $a^2$ is connected with it in this section.

Figure 11 is a section taken 160 micra below figure 10. The opening by which atrium $a^2$ communicates with an air-sac is cut through, and a small portion of the opening by which the atrium communicates with its *ductulus* is shown. Three air cells ($a^3$, $a^3$, $a^3$) which belong to the atrium bearing the same number are seen just at the left of atrium $a^2$. Only a small portion of the *ductulus alveolaris* which we have been considering remains, while the *ductulus* which first appears in figure 10 is shown cut through its entire length; also a small portion of an atrium and air-sac which are directly continuous with its axis. A very interesting series of air-spaces is seen on the left side of the section, and they furnish a good example of the necessity of serial sections in studying the architecture of the lung. These spaces belong to a still different system and are continuations of the *bronchiolus respiratorius* of which a small portion appears on the extreme left of the section.

The four sections which have been described and analyzed form a block which is 0.5 mm. long, 0.36 mm. wide and 0.6 mm. thick in its greatest measurements. In this block three *bronchioli respiratorii* and their subdivisions have appeared, one of which has been followed out to its ultimate ending in *ductuli alveolares,* atria, *sacculi alveolares,* and alveoli. Certain factors which still further aid in distinguishing the atria will be discussed in the following section.

The *tunica muscularis* of the trachea, which is made up of bands of smooth muscle, is continued into the bronchi, where it is often called the *Muscle of Reisseisen.* As the cartilaginous plates disappear from the walls of the bronchi the muscle increases in quantity. In the larger bronchi the muscle is arranged in the form of anastomosing bands, which have a direction nearly transverse to the long axis of the bronchus, but

FIG. 12.—Two views of a reconstruction showing the distribution of smooth muscle bands. *b.*: bronchiolus; *b. r.*: bronchiolus respiratorius; *d. al.*: ductulus alveolaris; *s. al.*: sacculus alveolaris.

nowhere do they form a complete sheet of muscle as, for example, in the walls of the small intestine. As the *bronchioli respiratorii* are approached the muscle bands take a more oblique course and have a wide, open mesh. This change of direction has an important significance.

In the preceding section it was seen that alveoli were present along the *bronchioli respiratorii* and that the same was true in regard to the *ductuli alveolares*. The openings leading into these alveoli are always situated in the open mesh of the smooth muscle; they could not be otherwise situated. It was also seen that both of these subdivisions of the bronchial tree take part in respiration. This necessitates the providing for a certain amount of movement.

The bronchi and bronchioli do not open and close along the main-stem bronchus like a fan, neither is their movement like that of a bellows. The latter consists of a widening of the angle at which the branches leave the main-stem bronchus; at the same time they become elongated and narrowed, and a slight rotation takes place. This movement is most marked in the lower lobes, where the amount of movement is the greatest, due to the expansion of the inferior aperature of the thorax and the descent of the diaphragm. It is to accommodate these movements that the change in the muscular network takes place; the muscle now takes the form of geodesic bands which prevent any tangential motion. In this way provision is made for the greatest amount of strength and at the same time permitting the greatest amount of extension and contraction (Fig. 12).

*Smooth muscle does not extend beyond the ductuli alveolares.* Nearly every investigator, who has studied the subject, has confirmed the statement, made by both Kölliker and Schulze, that no muscle is found beyond what is now termed a *ductulus alveolaris*. If sections which apparently show muscle in the walls of the air-spaces be followed through a series of sections it will be found that the muscle does not belong to the air-spaces, but to some division of the bronchial tree or to a blood-vessel.

At the distal end of the *ductuli alveolares* the muscle is arranged in the form of a sphincter about the openings leading

into the atria. This arrangement of the muscle about the atrial openings plays an important rôle in many cases of asthma by becoming strongly contracted during the asthmatic attack. Moreover, in the lungs of chronic asthmatic individuals the muscle bands throughout the lung are much thickened. In

Fig. 13.—Camera lucida tracing of a section of a primary lobule taken through the distal end of a ductulus alveolaris. *T.*: ductulus alveolaris; *A*, *A*, *A.*: three atria; *S.*: portions of two sacculi alveolaris. The broken lines indicate longitudinal sections of the smooth muscle in the walls of the ductulus. The stippled areas indicate transverse sections of the smooth muscle bands about the openings leading into the atria. The pulmonary veins are shown in solid black. The pulmonary arteries have stellate openings in them.

figure 13, which is a camera lucida tracing of a section through a primary lobule near the distal end of a *ductulus alveolaris,* the position of these bands about the atrial openings is shown; in some places the bands are cut transversely; in other places they are cut lengthwise, depending on the position of the opening leading into the atrium.

That elastic fibres run lengthwise along the bronchial tubes is an old and well-established fact. Some of the early investigators mistook these fibres for muscle, and described an inner longitudinal and an outer circular layer of muscle. These fibres give additional support to the walls of the bronchial tubes, especially where the muscle is deficient; they also take part in the extension and contraction of the bronchi and bronchioli during respiration. With the appearance of alveoli along the walls of a bronchiolus the elastic fibres no longer form a distinct layer, but are continued into the network of elastic fibres which are present in the walls of the air-spaces.

The normal lung contains *reticulum*. This is distributed throughout all portions of its framework. In the walls of the alveoli it encloses within its mesh the capillaries and forms the basement-membrane on which the pulmonary epithelium rests. It is especially abundant in the masses of lymphoid tissue that are situated in the angle formed by dividing bronchi, and in other situations where lymphoid tissue normally occurs.

Associated with the development of a tubercle there is an increased development of reticulum. Previous to the formation of giant cells but few fibrils are present; the giant cells, however, take no part in the formation of reticulum. Within a tubercle the reticulum forms a system of branching and anastomosing fibres which are everywhere present, and enclose within its mesh the giant cells, the endothelial cells and the lymphocytes (Fig. 14). This network of fibres is finer in the centre of the tubercle than at its periphery, where they show a tendency to be converted into collagenous fibres. In case the growth of the tubercle becomes arrested this actually occurs, and the tubercle is converted into a dense nodule of collagenous tissue.

There are, however, formations made up originally of single tubercles which have developed separately, but close to each other, which, by their fusion have formed larger or smaller masses called congolemate tubercles. With the coalescence of the single tubercles to form a conglomerate tubercle certain changes take place. It is no longer spherical in outline but is irregularly oval and lobulated. Its centre soon changes its char-

acter; the cells become necrosed and caseation gradually takes place. These changes often occur in the individual tubercles before fusion takes place.

If a section through a conglomerate tubercle, which has undergone caseation in its centre, be stained with haematoxylin and eosin, the caseation appears as a fine granular mass. The special stain for reticulum, however, shows that the caseated area is everywhere invaded with a network of fibrils, that is, the reticulum (Fig. 15).

Anatomically three types of tuberculous bronchopneumonia can be recognized. Each type presents a characteristic picture when stained for reticulum.

In type 1 the walls of the alveoli are recognized with difficulty, and there is no increase of reticulum in their walls. No reticulum is found within the alveoli themselves. The exudate which fills the alveoli undergoes an early and rapid caseation. Miliary tubercles are rarely present (Fig. 16).

In type 2 the walls of the alveoli are increased in thickness and they contain an increased amount of reticulum. The alveoli are filled with an exudate in which caseation takes place quite early. Miliary tubercles are present in considerable numbers. The reticulum shows a tendency to encapsulate the caseous areas without, however, extending into the alveoli themselves (Fig. 17).

In type 3 the walls of the alveoli are thickened; the alveoli themselves contain an exudate that consists largely of endothelial cells,[2] lymphocytes, and a few polymorphonuclear leucocytes, which are enclosed within a dense network of reticulum. The reticulum in the walls of the alveoli is increased in quantity and is directly continuous with the network within the alveoli. Because of these characteristics which differentiate this type from the two preceding types, I have designated it *reticular pneumonic tuberculosis*. The exudate in other types of pneu-

[2] These cells are also called "epithelioid;" "reticular;" "large mononuclear;" and "monocytes." They correspond to the "endothelial leucocyte" of Mallory. Until their embryonic origin has been definitely determined the nomenclature is bound to be confusing.

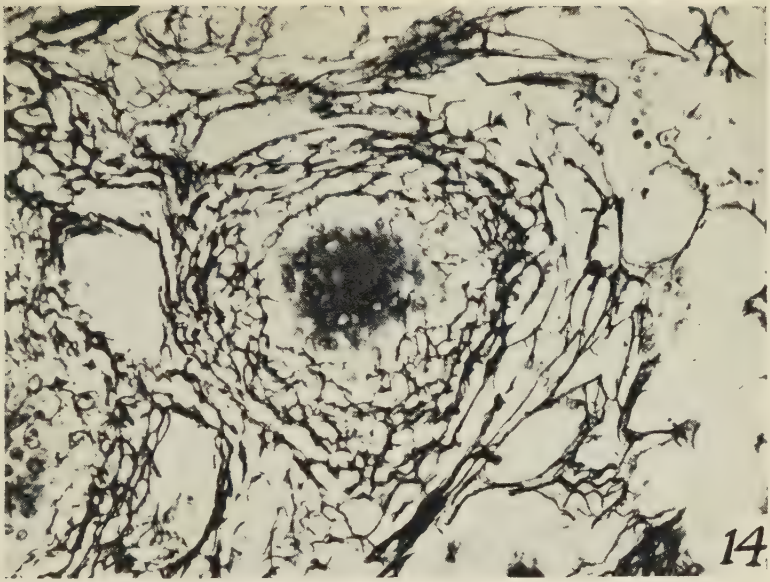



FIG. 14.—A single microscopic tubercle enclosed within a basket network of reticulum. Reproduced from the Amer. Rev. Tuberc., 1923, vii, 147.

FIG. 15.—A conglomerate tubercle, which has undergone caseation in its centre, showing the persistence of reticulum within the caseous centre. Reproduced from the Amer. Rev. Tuberc., 1923, vii, 147.

monia usually contains threads of fibrin, *never reticulum.* A small amount of fibrin may be present in this type also; but it is reticulum that predominates (Fig. 18). Miliary tubercles are present throughout the lung.

A rapidly progressive caseous tuberculosis, apparently the result of an overwhelming infection, is often called "acute phthisis," "phthisis florida," "galloping consumption," and other names too numerous to mention. This may involve a single lobe, the lobar form; or it may occur in a widely disseminated bronchopneumonic form. Both are characterized by a cellular exudate that fills the bronchi and alveoli, and rapidly undergoes caseous necrosis. The bronchopneumonic form is more frequent than the lobar, and corresponds to type 1, described above.

In the lobar form a single lobe may be affected or, rarely, nearly the whole of one lung. The affected lobe presents a complete consolidation. The cut surface may be smooth, or it may be finely granular. The outlines of the alveoli are recognized with difficulty. No tubercles are present and, except in the inflamed and thickened pleura, and the connective-tissue septa, there is no increase of reticulum. The onset of the infection has been too precipitate, and the extension too rapid, for the lung to show any signs of resistance. If the individual live long enough, the caseous mass may break down and extensive cavities be formed.

Reticulum plays an important rôle in pulmonary tuberculosis. If the infection is not a severe one, it is constantly trying to wall off infected areas by its conversion into collagenous fibres. Evidence of this is frequently found in miliary tubercles and in the walls of cavities. On the other hand, in the bronchopneumonic and lobar forms of caseous tuberculous pneumonia, in which the lung has been overwhelmed by the invasion of tubercle bacilli, reticulum is but slightly developed.

That reticulum is very resistant is shown by its persistence not only in purely tuberculous processes, but also in those cases in which pulmonary gangrene occurs as a concomitant of tuberculosis. In each instance it is fully as resistant as the elastic fibres.

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## EXPLANATION OF FIGURES

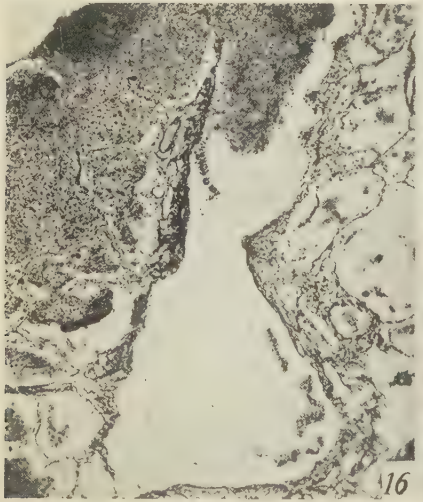
Fig. 1. Rossignol's diagram of the lung, 1846. *a:* two adjoining infundibula which have become fused; *b:* the next to the last bronchial division; *c:* a single infundibulum connected with a bronchial tube. Apparently the last division of the bronchial tree since *b* is next to the last division.

Fig. 2. Kölliker's diagram of the lung, 1852. *a, a:* two primary lobules (sacculi); *b, b:* air-cells or alveoli; *c, c:* the finest bronchial branches; these also possess air-cells.

Fig. 3. Waters diagram of the lung, 1860. *a:* terminal bronchial tube; *b:* its dilated extremity; *c, c, c:* individual air-sacs; *e, e:* openings leading into other air-sacs. The six air-sacs converge to a common centre.

Fig. 4. Schulze's diagram of the lung, 1871. *a:* terminal bronchial branch; *b, b:* infundibula; *c, c:* transverse sections of alveolar passages; *d, d:* longitudinal sections of alveolar passages.

Fig. 5. Kölliker's diagram of the lung, 1881. *br. r.:* bronchiolus respiratorious; *ag, ag.:* two alveolar passages, one of which terminates in an infundibulum. The openings in the walls of these various divisions lead into the alveoli.



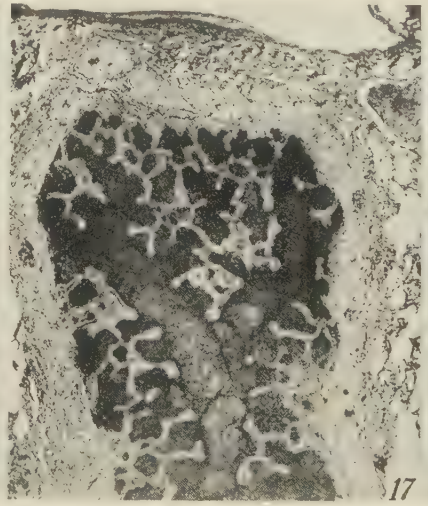


FIG. 16.—Type 1 of tuberculous bronchopneumonia. See text. Note the indistinctness of the alveolar walls within the caseous area, that there is no increase of reticulum in the pulmonary framework, and that there is no reticulum within the alveoli.

FIG. 17.—Type 2 of tuberculous bronchopneumonia. See text. Note the increased thickness and the increased amount of reticulum in the walls of the alveoli, the great amount of reticulum and of collagenous fibres which form the capsule about the pneumonic area, the absence of reticulum within the alveoli, and the presence of tubercles with giant cells. The upper boundary of the section is formed by a pulmonary vein, and the heavy band of collagenous fibres form part of its wall.

FIG. 18.—Type 3 of tuberculous bronchopneumonia. See text. Note the thickened alveolar walls in the lower left quadrant, the great increase of reticulum in their walls and the extension of the reticulum into the lumen of the alveoli.

FIG. 6. Author's diagram of the lung slightly modified from his 1892 diagram. *b.r.:* bronchiolus respiratorius; *d. al.:* ductulus alveolaris; *a, a, a:* three atria; *s. al., s. al., s. al.:* sacculi alveolares; *a. p., a. p.:* alveoli pulmonis.

FIG. 7. Camera lucida tracing from a section of a human lung in which a bronchiolus, two bronchioli respiratorii, a ductulus alveolaris, an atrium, and a sacculus alveolaris with its alveoli, are cut longitudinally. This section was taken 6 cm. below the pleura and formed part of a secondary lobule. The connective-tissue septa between the adjoining lobules forms the boundary of the section.

FIGS. 8–11. For full description of these figures see the text. Reference lettering is the same for all four figures. *b. r.*, bronchiolus respiratorius; *a, a, a:* portions of three atria; *s. al.:* sacculus alveolaris. The sections are bounded above by the pleura and on the right by a septum which separates two adjoining secondary lobules from each other.

FIG. 12. Two views of a reconstruction showing the distribution of smooth muscle bands. *b.:* bronchiolus; *b. r.:* bronchiolus respiratorius; *d. al.:* ductulus alveolaris; *s. al.:* sacculus alveolaris.

FIG. 13. Camera lucida tracing of a section of a primary lobule taken through the distal end of a ductulus alveolaris. *T.:* ductulus alveolaris; *A, A, A:* three atria; *S.:* portions of two sacculi alveolares. The broken lines indicate longitudinal sections of the smooth muscle in the walls of the ductulus. The stippled areas indicate transverse sections of the smooth muscle bands about the openings leading into the atria. The pulmonary veins are shown in solid black. The pulmonary arteries have stellate openings in them.

FIG. 14. A single microscopic tubercle enclosed within a basket network of reticulum.

FIG. 15. A conglomerate tubercle, which has undergone casecation in its centre, showing the persistence of reticulum within the caseous centre.

FIG. 16. Type 1 of tuberculous bronchopneumonia. See text. Note the indistinctness of the alveolar walls within the caseous area, that there is no increase of reticulum in the pulmonary framework, and that there is no reticulum within the alveoli.

FIG. 17. Type 2 of tuberculous bronchopneumonia. See text. Note the increased thickness and the increased amount of reticulum in the walls of the alveoli, the great amount of reticulum and of collagenous fibres which form the capsule about the pneumonic area, the absence of reticulum within the alveoli, and the presence of tubercles with giant cells. The upper boundary of the section is formed by a pulmonary vein, and the heavy band of collagenous fibres form part of its wall.

FIG. 18. Type 3 of tuberculous bronchopneumonia. See text. Note the thickened alveolar walls in the lower left quadrant, the great increase of reticulum in their walls and the extension of the reticulum into the lumen of the alveoli.

# THE RECOVERY PROCESS AFTER MUSCULAR EXERCISE IN MAN[1]

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## MUSCULAR FATIGUE

THE isolated muscle stimulated in air, or in Ringer's solution saturated with air, with more than a certain frequency, rapidly fatigues: stimulated infrequently it may remain alive for several weeks. If maintained in Ringer's solution saturated with oxygen, it may be stimulated once every ten seconds for several days; with very frequent stimulation it is completely exhausted in a few hundred twitches. Left without oxygen, it never recovers from this fatigue; in a few hours it goes into rigor and dies. Left in oxygen, however, it completely recovers, its contracture disappears, and it is ready to function again as well as ever. Moreover, in oxygen it will rapidly reach a steady state in which breakdown in activity is balanced exactly by restoration in recovery. This steady state in oxygen is of peculiar importance when we come to study prolonged human muscular activity.

## LACTIC ACID

Such observations on the isolated muscle led to a study of lactic acid—its accumulation in activity, its removal in recovery. Fletcher and Hopkins's experiments, published in 1907, have proved the basis of all subsequent advance in muscle physiology. In a muscle left at rest in oxygen the lactic-acid concentration never increases and may decrease; in a muscle left in air, it increases slowly; more rapidly, however, in inert gases, such as hydrogen or nitrogen. If a muscle be subjected to some harmful influence, such as that of mechanical injury, coal-gas, alcohol, or chloroform, its lactic-acid content increases rapidly. This anærobic production of lactic acid is increased by a rise of tem-

[1] Lecture delivered October 25, 1925.

perature, an effect which culminates in an almost explosive production of lactic acid during heat rigor, which occurs in such an animal as the frog, at about 35.5° C. When a muscle is initially fatigued and then left in oxygen, its lactic-acid concentration continually falls over a period of hours—this is the recovery process—and the muscle becomes as capable as before of reacting to a stimulus. In such a case—regarded from the standpoint of the intact animal—the oxygen supply is grossly inadequate, owing to the slowness of its diffusion through the muscle substance from outside. The recovery process, in the presence of an adequate supply of oxygen, as in man after moderate exercise, or in the isolated muscle saturated with oxygen after a single twitch, is far more rapid. This abolition of lactic acid during the recovery process does not appear to diminish the total amount of it which can be obtained later from the same muscle by stimulation, by mechanical injury, or by heat rigor; either it is not oxidized but rather restored to some previous position in the chemical architecture of the muscle, or there is some limit to the lactic-acid concentration set by its own presence there, for example, by the rise in hydrogen-ion concentration which it causes. Both explanations are essentially true.

These experiments of Fletcher and Hopkins, which have been fully and widely confirmed since, were obviously of fundamental importance in the study of muscle. They were the starting-point of my own investigations, as they were of Meyerhof's. They clearly opened up all kinds of problems—what happened to the lactic acid when it was removed in recovery? How long did the process take when the oxygen supply was inadequate? Was the lactic acid the essential link in the chain of muscular contraction, or only a by-product to be got rid of as soon as possible? Many of these questions have since been answered.

### THE HEAT-PRODUCTION OF MUSCLES

There is a special advantage in studying the heat-production of muscles. The first and most important factor in a machine is the amount of energy which it uses, and the heat-production is

a measure of this. In studying muscles, moreover, *heat* measurements can be made without injury to the tissue, continuously while the processes inside it are going on. To study the *chemical* changes in a muscle requires us to destroy it each time we make an observation. The sensitivity, moreover, of the thermo-electric method is so great that it is possible to examine, during and after a single twitch, the process which can be studied by chemical means only after severe activity.

### RIGOR MORTIS

When a frog's muscle is warmed to 35° C. or more, or is exposed to chloroform vapor, it goes rapidly into rigor, the only chemical event known to accompany this process being the production of lactic acid, followed by the effect of the acid in producing stiffness or coagulation of the muscle substance. This is the same process as occurs, more slowly, in anærobic survival at lower temperatures. The heat liberated during rigor is one to two calories per gram of muscle. Seeing that no chemical events are known to accompany the onset of rigor except the lactic-acid production, it was natural to attribute this liberation of heat to the (then) unknown process by which the lactic acid also is liberated. The heat produced per gram of lactic acid was estimated roughly in 1912 as being about 370 calories. Recent and much more accurate measurements by Meyerhof have confirmed this value. Peters, in 1913, found that the heat liberated in the absence of oxygen, during stimulation to complete fatigue, is about 0.9 calories per gram of muscle. Rigor in the unstimulated muscle gave about 1.7 calories. Stimulation of fresh muscles leads to the liberation of about 0.2 per cent. of lactic acid; rigor to one of about 0.4 per cent.; stimulation followed by rigor also to about 0.4 per cent. Hence it was obvious that lactic acid and heat formation run parallel to one another, and that rigor and anærobic activity involve the same chemical change—namely, the production of lactic acid from some precursor with the liberation of about 370 calories for every gram of acid set free.

### THE ORIGIN OF LACTIC ACID

This determination of the heat production associated with the formation of 1 gram of lactic acid, either in rigor or in the anærobic activity of muscle, enables us to decide with fair certainty what are the chemical reactions associated with contraction, and from what substance the lactic acid arises. Now it is probable on other grounds that glycogen is the precursor of lactic acid. The heat of combustion of dissolved glycogen $(C_6H_{12}O_6)_n$ has been determined by Slater as 3836 calories, that of lactic acid ($C_3H_6O_3$) by Meyerhof as 3601 calories per gram; the formation, therefore, of lactic acid from glycogen is accompanied by the liberation of 235 calories per gram. Now this lactic acid never occurs as such, to any appreciable degree, inside a muscle; it is always there neutralized in the form of sodium or potassium lactate. There are reasons for believing that the neutralization takes place at the expense of a sodium protein salt, the alkali-protein substance of the muscle itself. According to Meyerhof, the heat of this neutralization is about 135 calories, so that the total heat associated with the formation of lactic acid from glycogen and its subsequent neutralization would be 370 calories. This is precisely the amount of heat set free when 1 gram of lactic acid is formed inside a muscle during activity or rigor. It is obvious, therefore, that in the initial processes of contraction and relaxation—*i.e.*, as distinguished from recovery —no chemical change of any importance occurs except the formation of lactic acid at the expense of glycogen and its subsequent neutralization.

That glycogen is, indeed, the precursor of lactic acid in the muscle is shown by the direct determinations of Meyerhof, which have been confirmed in Hopkins's laboratory. During anærobic activity, or survival, glycogen disappears from the muscle and lactic acid appears in exactly equivalent amount; $CO_2$ is driven out by the lactic acid from the bicarbonate present in the muscle; heat is liberated proportionally to the lactic acid. No change, however, is observed in the fat, and the lactic acid maxima are in exercise 0.25 to 0.4 per cent., in rigor 0.4 to 0.7

per cent. The reaction is apparently a self-inhibited one; it is stopped by the rise of hydrogen-ion concentration produced by the lactic acid and may be caused to go further by the presence around the muscle of alkali and phosphates. Presumably, the complete failure to contract, produced by muscular fatigue, is due to the same cause.

### BUFFERS IN MUSCLE

The accommodation in the muscle of these enormous quantities of lactic acid requires some special consideration. It has been stated that during activity a muscle becomes acid; so it does, but only slightly. Were the acid free inside it the hydrogen-ion concentration would rise thousands of times as the result of a few contractions. Ritchie, in 1922, showed, by an electrometrical method, that no appreciable change in the hydrogen-ion concentration of a muscle occurs after several seconds' tetanus; the acid, therefore, is neutralized. In blood we know that there are various alkaline buffers—bicarbonate, phosphate, and particularly the sodium or potassium salt of hæmoglobin. These react with an added acid, forming a neutral salt and the undissociated buffer acid. So it is in muscle. The total amount of bicarbonate and phosphate is insufficient to neutralize all the acid formed in exercise, and it has been shown by Meyerhof that the main part of the neutralisation must occur as the result of combination of the acid with the sodium protein salt of the muscle tissue itself. The amount and efficiency of this buffer constituent of the muscle are the chief factors enabling the muscle to stand high concentrations of acid—that is, to tolerate a high degree of activity.

### MYOTHERMIC EXPERIMENTS

Our knowledge of the time course and the magnitude of the events which occur during and after muscular activity has been derived largely from a study of the heat-production. The thermo-electric method is a highly sensitive one and enables us to record and analyze the heat-production associated with a single muscle twitch. It is essential to keep the muscle in its

chamber far removed from any external temperature disturbances; this is done by sinking it under water maintained at a constant temperature in a large double-walled vacuum flask. Connected to a sensitive galvanometer the thermopile registers its changes of temperature on a revolving drum carrying photographic paper. When the muscle is stimulated, its temperature rises and the galvanometer deflects, the moment of stimulus being recorded on the record. The deflection reaches a maximum comparatively slowly, owing to lag in conduction of heat into the thermopile and inertia of the galvanometer system. It then moves slowly back towards its original zero, owing to gradual equalization of temperature along the thermopile and loss of heat by conduction to outside. The curves by themselves do not tell us directly much about the time course, or the absolute magnitude, of the liberation of heat; it is necessary to analyze them by a somewhat laborious numerical technique.

### THE RECOVERY HEAT-PRODUCTION

The results of the analysis are as follows: A large amount of heat is liberated during the onset and maintenance of contraction and again during relaxation. It is natural to associate the first production of heat with the formation of lactic acid from glycogen, the second with the neutralization of lactic acid. It is soon found, however, that the heat-production is not finished when the muscle has relaxed; the deflection of the galvanometer when the muscle is in oxygen returns only to zero, far more slowly than in a dead muscle warmed suddenly at the beginning. This can be due in the live muscle only to a continued formation of heat for long periods after the contraction

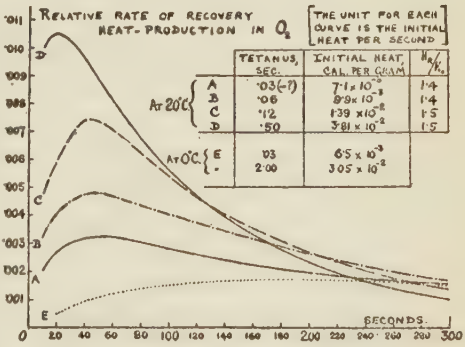
RELATIVE RATE OF RECOVERY HEAT-PRODUCTION IN $O_2$
THE UNIT FOR EACH CURVE IS THE INITIAL HEAT PER SECOND
TETANUS, SEC.
INITIAL HEAT, CAL. PER GRAM
$K_2/K_1$
At 20°C
A ·03(-?) 7·1 x 10⁻² 1·4
B ·06 8·9 x 10⁻² 1·4
C ·12 1·39 x 10⁻¹ 1·5
D ·50 3·81 x 10⁻¹ 1·5
At 0°C.
E 03 6·5 x 10⁻³
2·00 3·05 x 10⁻²
SECONDS
0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300

FIG. 1.—Recovery heat production of frog's muscle in oxygen.

is over, this heat maintaining the temperature of the thermopile and the deflection of the galvanometer. The recovery heat-production (Fig. 1), as it is called, goes on for 10 to 20 minutes at room temperature; at 0° it may be apparent nearly an hour after the stimulus. It is clearly concerned with the oxidative removal of lactic acid, which was found by Fletcher and Hopkins to occur in oxygen after activity. It is not affected, however, by diffusion, since the muscle contains, dissolved in its own substance, more than enough oxygen to account for the small amount of heat actually liberated in recovery from a single short contraction. We do not know in detail what the events are which occur during this restoration process. The curve, however of the recovery heat-production represents the thermodynamic outline of the recovery process as a whole.

### FACTORS AFFECTING THE SPEED OF RECOVERY

The recovery heat-production is affected by a variety of factors. A rise in hydrogen-ion concentration diminishes its rate, while leaving its total magnitude unaltered. A large decrease in the rate of recovery may be produced by a comparatively small rise in the hydrogen-ion concentration inside the muscle fibre. This is analogous to the effect produced by hydrogen-ion concentration on the rate of autoxidation of cystein or glutathione, or of the oxidation of fructose in the presence of phosphates. The rate of recovery is largely increased by a rise of temperature, diminished by a fall.

### CHEMICAL EVENTS IN RECOVERY

In a muscle kept in oxygen no lactic acid ever appears; lactic acid already formed disappears; oxygen is used and carbon dioxide is produced in excess of the resting values. The excess $CO_2$ is equal to the excess oxygen—*i.e.*, the R. Q. of recovery is unity. The restoration process, therefore, in the isolated muscle involves the oxidation of something with the general formula of carbohydrate. There is no sign of change in the fat inside the muscle; heat, as we have seen, is liberated and—

most important of all—glycogen has been shown by Meyerhof to be re-formed.

From the oxygen used and the $CO_2$ produced we may calculate the carbohydrate oxidized. The amount of glycogen re-formed, plus this amount of carbohydrate oxidized, is shown experimentally to be exactly equal to the amount of lactic acid which has disappeared. It would seem, therefore, that the lactic acid is mainly or entirely re-formed into glycogen during recovery, but that a certain amount of energy (obtained from the oxidation, either of a part of the lactic acid, or of carbohydrate—it is impossible to say which) is necessary in order to force the reaction backwards. In the anærobic process of activity—the process producing fatigue—glycogen becomes sodium lactate with the evolution of 370 calories per gram of lactic acid. In the recovery process the reaction is reversed, sodium lactate becomes glycogen, and 370 calories, therefore, of energy must be absorbed. This in itself would lead to a cooling of the muscle. Actually, Hartree and I have found that in the process of recovery there is an extra oxidative heat-production almost exactly equal to the total anærobic heat-production. The total recovery heat, therefore, is 370 calories, so that the total energy liberated in recovery is 740 calories. The heat of combustion, however, of dissolved glycogen is 3836 calories. Hence, in the complete cycle by which one gram of glycogen becomes one gram of lactic acid and then is restored from the acid, the fraction $740/3836 = 0.193$ gram of glycogen must be oxidized to provide the necessary energy. The initial process of contraction is forced backwards by the energy liberated in this oxidation. This reversal is the recovery process.

### "EFFICIENCY" OF RECOVERY

The ratio of the lactic acid removed during recovery to the lactic acid, or carbohydrate, oxidized to provide the necessary energy for its removal, may be named the "efficiency of recovery," or, as Meyerhof calls it, the "oxidative quotient"; it appears to be about 5 : 1—five molecules are removed for every one oxidized. It has proved possible on man also to make obser-

vations of the efficiency of recovery; they have led to the same values as those obtained on isolated muscle.

### LACTIC ACID IN MAN

It is a common observation that after exercise breathing is deep and rapid—that recovery is necessary. The same process must occur as in the isolated muscle, complicated in this case by the presence of the respiratory and circulatory mechanism. It can be shown that lactic acid occurs in human muscles as it occurs in isolated amphibian muscles. It had not been realized, however, till recently, how extensive are the changes connected with the lactic acid which is produced in the human body during exercise. When an athlete is running as fast as he can, about 5 grams of lactic acid per second are being liberated in his muscles. There are several signs of this lactic acid in the body. It occurs in the urine after prolonged hard exercise. In the blood of a resting man there is 0.01 to 0.02 per cent; after exercise it may exceed 0.10 and even attain 0.20 per cent. This may be estimated directly by chemical analysis, or it may be inferred, as Barcroft did, from the change which it produces in the oxygen dissociation curve. There is a very high respiratory quotient during, and especially just after severe exercise; the lactic acid raises the hydrogen-ion concentration of tissues and blood; this stimulates the respiratory centre, with the result that $CO_2$ is eliminated by the lungs. In a later stage of recovery correspondingly the respiratory quotient falls to very low values as the lactic acid is removed. The chief sign, however, of the amount of lactic acid present in the body after exercise is the magnitude of the so-called "oxygen debt."

A muscle which has been active requires oxygen to carry out the combustions needed to provide the energy for the restoration process. The oxygen used in recovery is a measure of the magnitude of that process—that is, of the amount of lactic acid removed. Assuming an efficiency of recovery—an "oxidative quotient"—of 5.2 : 1, one litre of oxygen consumed after exercise is equivalent to 7 grams of lactic acid removed. The greatest oxygen debt hitherto recorded is one of nearly 19 litres, which

corresponds to a concentration of lactic acid not far from 0.35 per cent. in all the muscles of the subject; he was well-nigh exhausted.

### STUDIES OF OXYGEN INTAKE

The greater part of our knowledge of the events occurring in human muscular exercise has been arrived at by studies of the gaseous metabolism. The measurement of the oxygen intake is possible in various ways. The method adopted in most of the experiments now to be described is that of the Douglas bag, in which the subject expires into a large bag carried on his back or in front of him, and the expired gases are analyzed and their volume measured. It is possible to use this method when walking, running, riding a bicycle, even swimming, and to plot the time course of the oxygen consumption in intervals as short as half a minute.

### THE "STEADY STATE"

I spoke earlier about the steady state of an isolated muscle contracting in an atmosphere of oxygen; in such a muscle there is a balance between breakdown and recovery. The recovery process is more rapid when the total amount of breakdown causing it is greater; lactic acid is a sensitive "governor" of oxygen usage. Hence, if we start with a given *rate* of breakdown, as when a man suddenly begins to run at a given speed, the rate of recovery, starting from zero, has to work up gradually until it balances the rate of breakdown. It is necessary, moreover, in the intact animal for the circulation and respiration to work up also. In a subject starting to run, having been previously at rest, the oxygen intake rises to its full steady value in a period of about two to two and a half minutes. If the running be continued at a constant speed the oxygen intake also remains constant thereafter—or almost constant—till the end of the exercise; the subject is in what we may call a "steady state." This state corresponds to a constant concentration of lactic acid in the muscles, constant owing to a balance between formation and removal. As the exercise, however, is continued, the lactic acid present in the active muscles gradually passes, by diffusion, into

the blood and thence to other parts of the body. The oxidative removal of the lactic acid formed in one muscle may in this manner occur in other distant muscles, by its passage, *via* the blood, from the one to the other. As Barr and his colleagues have found, the venous blood from a resting muscle, during the vigorous activity of others, may contain *less* lactic acid than the arterial blood coming to it. Indeed, in this sense it is possible to recover in one's arms from exercise taken in one's legs! My friend, Professor Meyerhof, informs me that in recent experiments he has succeeded in establishing the formation of glycogen in frogs' limbs perfused with Ringer's solution containing sodium lactate, but only in the presence of oxygen, as was to be expected. This may be an important factor in recovery from vigorous exercise taken by isolated portions of the muscular system; it cannot, however, have much effect in severe general exercise.

An apparent "steady state" may occur, during muscular exercise, in which the oxygen intake is constant, which is not, however, really "steady" at all; there is a continual onset of fatigue. The oxygen intake may attain its maximum and remain constant merely because it cannot go any higher owing to the limitations of the circulatory and respiratory systems. Such a condition causes an accumulation of lactic acid in the muscle and can end only in complete exhaustion.

### RECOVERY

When the exercise terminates the lactic acid in the muscles must be removed and oxygen must be taken in to provide the necessary energy. The lag in the oxygen intake at the beginning of exercise, during which the lactic acid was concentrating, must be compensated by an extra intake afterwards when it is diminishing again. After a short bout of moderate exercise the oxygen intake falls, in a period of five to ten minutes, to its resting value. After severe or prolonged exercise it may be eighty minutes before the oxygen intake is back again. The *total* amount of oxygen used in the recovery process may be measured by collecting the whole of the expired gases over any desired interval,

by an estimation of the total oxygen used in that interval, and subtraction of the amount of oxygen which the subject would have used had he remained throughout at rest.

"OXYGEN REQUIREMENT"

It is desirable here to introduce a new term—namely, the "oxygen requirement" of exercise. If the exercise be very mild, then the oxygen requirement may be met by the actual

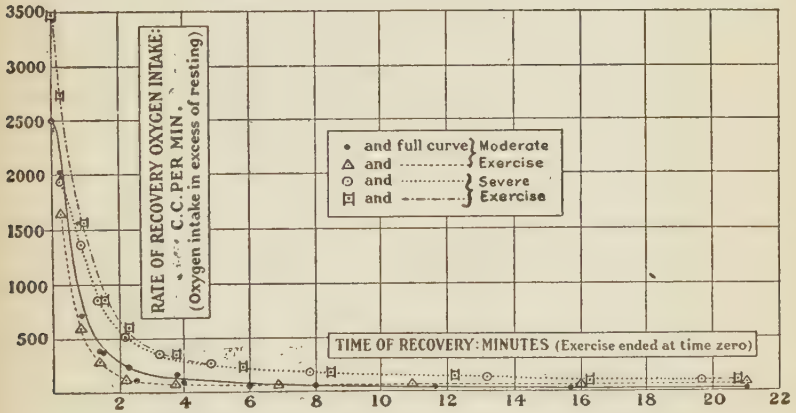


FIG. 2.—Recovery oxygen intake moderate and severe exercise.

oxygen intake, once the steady state has been attained (Fig. 2). If, however, the exercise be severe, the oxygen requirement cannot be met, even when the heart and lungs have attained their maximum activity; if the exercise be persisted in, the body necessarily incurs what we may call an "oxygen debt." Were it not for the fact that the body is able to obtain its energy in this way, by using its necessary oxygen afterwards, severe exercise would be impossible in man. We should never be able to run upstairs, or to run on the flat at more than eight or nine miles an hour. We can do so only because Nature has provided us with an arrangement, like an accumulator which can be run down at a very high rate, as in starting a car, and then must be recharged slowly afterwards.

The oxygen requirement of a given type of exercise, or of a

given isolated movement or series of movements, can always be measured. It may be much higher than any possible oxygen intake. If the movement be carried out for a short time, and the total oxygen used (in excess of the resting value) *during it, and in recovery from it,* be ascertained, then the total oxygen so found is the requirement of that piece of exercise. In this way it is possible to study the energy requirement of even the most violent and discontinuous movements. To take an example, Lupton measured the optimum speed and the maximum efficiency of the process of climbing a staircase. The subject ascends the staircase once, at any required speed varying from very slow to very rapid. The oxygen used during the

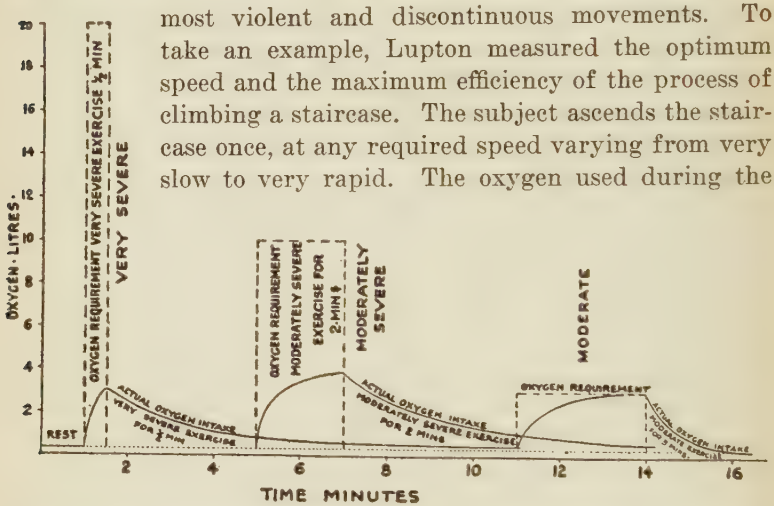
OXYGEN · LITRES ·
TIME MINUTES
REST
OXYGEN REQUIREMENT VERY SEVERE EXERCISE ½ MIN
VERY SEVERE
ACTUAL OXYGEN INTAKE VERY SEVERE EXERCISE FOR ½ MIN
OXYGEN REQUIREMENT MODERATELY SEVERE EXERCISE FOR 2 MIN
MODERATELY SEVERE
ACTUAL OXYGEN INTAKE MODERATELY SEVERE EXERCISE FOR 2 MINS
MODERATE
OXYGEN REQUIREMENT
ACTUAL OXYGEN INTAKE MODERATE EXERCISE FOR 3 MINS

FIG. 3.—Oxygen requirement and oxygen intake for moderate, moderately severe and severe exercise.

climb and in the succeeding twenty minutes, after subtraction of the resting value for the same time, gives the oxygen consumption produced by the exercise. This decreases at first as the speed increases, and then increases again, showing that there is indeed an optimum speed.

The existence of an optimum speed does not occur in all types of exercise. In running the oxygen *requirement* increases continuously as the speed increases, though the actual oxygen *intake* reaches a maximum beyond which no effort can drive it. Once the oxygen requirement is larger than the maximum oxygen intake, a steady state is no longer possible, and fatigue ensues more or less rapidly.

### "OXYGEN DEBT"

Recently we have made a number of estimations of the "oxygen debt" at the end of exercise. The most striking are those (a) in which 23 secs. of exercises led to a debt of 8.7 litres,[2] (b) running for 33 minutes at a medium speed only of 7.9 litres, and (c) "standing running" with extreme violence for 4 minutes of 18.7 litres. The larger values mean enormous amounts of lactic acid accumulated in the body. This acid, of course, is neutralized inside the muscle, and the hydrogen-ion concentration there never rises very far so long as the amount of alkali is adequate. It would seem probable that the limit to which it is possible to press a muscle is determined by the amount of alkali within it capable of neutralizing the acid product of its activity.

### $CO_2$ ELIMINATION AND RETENTION

This sudden production of acid is the explanation of the large amounts of $CO_2$ that come off during and immediately after exercise. When the lactic acid is removed in recovery, the same quantity of $CO_2$ must be retained as was originally eliminated; sodium lactate must become sodium bicarbonate again. Consequently, we find very low respiratory quotients in the later stages of recovery. The $CO_2$ retained is a measure of the lactic acid got rid of. The oxygen used in recovery is a measure of the carbohydrate (or lactic acid) oxidized. The comparison of the two enables us to determine in man the "efficiency" of recovery. We find that only a small fraction of the lactic acid has been oxidized, the value determined for the "efficiency" of recovery is almost identical with that found in the case of isolated frog's muscle.

The fluctuations in the respiratory quotient, resulting from these lactic acid changes, are very striking, and the respiratory quotient returns to its original value only after complete recovery has occurred. Indeed, the return of the respiratory quotient to its normal resting value is one of the best indications of the completeness of recovery. After moderate exercise of short dura-

[2] More striking still, a first class sprinter recently acquired an oxygen debt of 7.8 litres in about 13 secs.

tion the return is rapid, after severe and prolonged exercise it is slow. Even after the most severe exercise, however, the respiratory quotient will be found to have returned to its resting value after 80 minutes, a duration of recovery which is confirmed, both by studies of the oxygen intake and by the direct measurement of lactic acid in the blood.

### DIRECT MEASUREMENT OF LACTIC ACID

During and after exercise lactic acid occurs in the blood; during the steady state of continued exercise this lactic acid accumulates to a constant concentration characteristic of the violence of the exercise. The rate of recovery, given an adequate supply of oxygen, is proportional roughly to the square of the concentration of lactic acid in the active muscle. The lactic acid is a sensitive governor of oxidation. After moderate exercise, when the oxygen supply is adequate, recovery is rapid. The lactic acid rapidly disappears from the active muscles. After severe exercise, however, the lactic acid in the muscles cannot fall very rapidly owing to the inadequacy of the oxygen supply. It diffuses out, therefore, into the blood and all round the body. It may reach its maximum in the blood some minutes after it has attained its maximum in the muscles. Hence, the maximum in the blood is considerably lower than in the muscles. At the particular moment, however, when it is at its maximum in the blood, we may be sure that it is the same in the muscles; equally it is certain to be the same in both at the end of recovery, when both are becoming constant. It is possible, therefore, by a direct analysis of lactic acid in the blood to ascertain the concentration in the muscles at two moments: (1) about ten minutes after exercise, and (2) towards the end of recovery. By such direct analyses it is possible to determine directly—if somewhat roughly—the absolute amount of lactic acid which has disappeared from the soft tissues of the body, which are in intimate contact with the blood, in the recovery process. This may be compared, as in the case of the lactic acid calculated from the $CO_2$ retention, with the oxygen used in that removal, and it is found, as in the frog's isolated muscle, that the acid is not oxidized; only about

one-fifth of the oxygen is used which would have been required had all the lactic acid been broken down to $CO_2$ and water.

### THE RECOVERY OXYGEN INTAKE

After moderate short-lived exercise there is only a single rapid phase of recovery, as shown by the fall of oxygen intake following such exercise. The oxidative process in this case is concerned only with the lactic acid present in the active muscles themselves. After prolonged severe or violent exercise, however, the same method shows decisively that there are two phases. The first, as before, is that in which the lactic acid still present in the muscles passes through the recovery mechanism; the second is that in which the lactic acid which, owing to the severity of the exercise, has escaped into the blood, and thence to distant corners of the body, has slowly to be dealt with, as it can be induced to return, by diffusion, to the muscles. The first phase occupies, in normal man, only 4 to 10 minutes; the second phase, when it occurs, is complete in 80 minutes.

### FACTORS AFFECTING SPEED OF RECOVERY

The speed of the recovery process depends in a healthy man on the oxygen supply to the active muscles. This is limited by two factors: first, the total oxygen that can be taken through the lungs into the blood and circulated by the heart; second, the local circulation to the part involved. Many observations have been made of the oxygen intake of a man undergoing various forms of severe muscular exercise. The highest value recorded while breathing air is 4.2 litres per minute; breathing 50 per cent. oxygen very nearly six litres has been reached. The only possible primary reason why more oxygen can be taken in, when breathing oxygen, is that the blood is not completely saturated during very severe exercise when breathing air; the greater saturation, however, of the coronary blood, when breathing oxygen, may then result secondarily in greater cardiac activity. The magnitude of the oxygen intake is determined mainly by the capacity of the heart, partly by the capacity and diffusion constant of the lungs. Another factor determining the speed of the recov-

ery process is the local supply of blood to the muscles actually involved in the work. Lindhard showed that in such exercises as holding the body with arms bent, on rings in a gymnasium, the blood supply to the active muscles of the arm is almost completely cut off. The whole of the oxygen required had to be used after the exertion was over. In running the supply of blood to the active muscles is particularly easy; they are never rigid and their movements are rapid. Consequently a high value of the oxygen intake may be attained.

The values of the oxygen intake during severe exercise allow a striking—if approximate—calculation of the output of the heart. Assuming—as was the case in the subject who consumed 5.9 litres of oxygen per minute—an oxygen capacity of 21 cc. per 100 cc. of blood, and an average utilization coefficient of 75 per cent. (a value as high as has ever been recorded), then the actual oxygen taken in must have required 37.5 litres of blood per minute to carry it. This amount of blood has been ejected from the heart twice, from the right side once, and from the left side once; altogether, therefore, 75 litres of blood—about 17 gallons, about 120 times the heart's own volume—have been pumped out in every minute. Even assuming a utilization coefficient of 100 per cent., 56 litres (12½ gallons) of blood must have been pumped together from the two sides of the heart. Compare these volumes with the amount of water which a bath tap can pour out in a minute, even when turned full on. In such exercise the heart-rate—recorded with a string galvanometer—is about 180, which means that each side of the heart ejects some 200 cc. per beat. It is difficult to believe the inevitable numerical deductions from quite simple and accurate experiments.

The limitations of the oxygen supply affect the recovery process mainly during the continuance of exercise. All muscular oxidation is recovery oxidation; even if it occurs during exercise, each element of oxidation must be regarded as supplying energy for the recovery from a previous element of the exercise. Rapidly, however, when exercise ends, the conditions alter: the oxygen supply becomes adequate, and even the breathing of a 50 per cent. oxygen mixture will not now affect the velocity of the

recovery process. The speed of the oxidative cycle depends now on more subtle chemical and physico-chemical factors. What they all may be we do not know; that they are, however, important factors in a man's capacity for severe exertion and rapid recovery cannot be questioned. Without a most effective oxidative mechanism Nurmi could scarcely have broken the world's record, both for 1500 and for 5000 metres, with only a short interval between them, in the recent Olympic games. We may, however, *guess* at what they are. It will be interesting to see how far they depend on training, age, condition, how far they are universal.

In the isolated muscle the speed of recovery depends on the hydrogen-ion concentration. Deep breathing, therefore, sweeping away the $CO_2$ and keeping the body alkaline may be important for quickness of the recovery process. It may depend also upon the presence of oxidative catalysts. Recent work by Hopkins has shown the presence in tissues of a di-sulphide compound, glutathione, which acts practically as such an agent. The discovery of similar substances has been made independently by Meyerhof. Recent work in Hopkins's laboratory has shown that hæmoglobin has a distinct catalysing power in the oxidation of unsaturated fats. The dyspnœa so often associated with anæmia may in some cases be due to the absence, not only of an adequate oxygen supply, but of an adequate amount of some oxidizing catalyst. Recent work by Warburg and his colleagues has shown that many forms of biological oxidation probably occur as surface phenomena, catalysed by iron atoms embedded in the surface; the absence of the iron, or its removal from activity by physical or chemical means, will hinder or prevent oxidation, as also will displacement of the oxidisable bodies, by narcotics or other substances. Imperfect metabolism of iron, excretion of it, its combination with abnormal metabolites, the displacement of the bodies to be oxidized from the oxidizing surfaces, might all lead, therefore, to an imperfect and slow recovery from exertion, to dyspnœa associated with exercise. This is guesswork, but at present we can do nothing but guess—and test our guesses.

APPLICATIONS

All of us can suffer from dyspnœa and all of us can exhaust ourselves rapidly; the finest athlete in the world may be incapable of movement within 60 seconds from the beginning of a race. The same degree, however, of exhaustion may be produced by a much lower level of exercise, no more rapidly but just as effectively, in a person of lower oxidative faculty or with poorer buffers in his tissue. Athletes and healthy men, however, give us the cleanest experiments. If one took a patient from the hospital and made him work till he could barely move, one could never be sure (a) that he had really driven himself to the limit—it requires an athlete to know how to exhaust himself; (b) that one would not kill him; and (c) what the cause of his stopping was. With young athletic individuals one may be sure—if one knows one's men—that they really have gone "all out," moderately certain of not killing them, and practically certain that their stoppage is due to oxygen-want and to lactic acid in their muscles. Quantitatively the phenomena of exhaustion may be widely different, qualitatively they are the same, in your athlete, in your normal man, in your dyspnœic patient. You can only *observe* your patient, but you can *experiment* with your athlete; you can "try out" on him the facts and theories you have reached with frogs. I shall return to my frogs, you to your patients, but I shall be more than glad if you can share my faith that even such experiments as these may lead, in the end, to the alleviation of human suffering. After all, it is a much longer trail from a small portion of a frog in a vacuum flask to a young athlete on a running track, than it is from him to a patient in a hospital. Faith in the consistency of Nature has been rewarded on the one trail; it can scarcely fail to be rewarded on the other.

# TEMPERATURE AND IMMUNO-CHEMICAL REACTIONS [1]

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THE chemical nature of the substances with which we work in the science of immunity is as yet quite unknown to us and for the present only such important researches as those of Avery, Heidelberger and their collaborators can vaguely descry the hope of any progress in this domain. But at the moment our immuno-chemistry must be exclusively based upon the knowledge of the action of these unknown substances.

As a result of this uncertainty, one is often inclined to speak of "functions." When one, for instance, says "complement," one often means "the capacity of serum to act as a complement." This feeling of uncertainty naturally becomes greater when it is a question of quantitative determinations.

The necessity of establishing a measure for the phenomena one meets with in the science of immunity has from the very first been recognized; Behring, and in particular Ehrlich, have done a great amount of work in the working out of methods of measurement for the strength of diphtheria antitoxin. On the other hand, it was long before it was acknowledged that the phenomena of immunity were subject to the same laws as those familiar in the other natural sciences. When, for instance, Arrhenius and I, some twenty-odd years ago, showed that the laws of physical chemistry could be applied to toxins and antitoxins, this idea was considered strange and awakened an opposition which nowadays is incomprehensible. It must, however, be remembered that to most medical men of that time the many new discoveries which had been made one on top of another at

[1] Lecture delivered November 22, 1924.

the end of the previous century appeared as something so remarkable and peculiar that they were more inclined to attribute the phenomena of immunity to forces which were quite apart from those hitherto known than to refer them to the domain of the exact natural sciences.

What also contributed to some degree in bewildering the ideas was the fact that during the rapid rise which colloid chemistry began to take about the beginning of this century, there was often a tendency to regard the reactions of colloidal substances as being something quite apart, different from those of other known substances. There was a general inclination, when one could not interpret the laws for an immuno-chemical process, to call it "colloidal" and to feel satisfied with this explanation.

You will be aware that opinions in this respect have undergone a complete change after the fundamental researches by investigators such as *Hardy, S. P. L. Sörensen, Proctor, Wilson* and others. There is particular reason for mentioning *Jacques Loeb's* splendid work of most recent years, in which he seeks to penetrate more deeply into the colloidal reactions of the proteins. It is a great loss to our science that he should be taken away in the middle of this important work.

Thus there is no more reason for adopting another view of the processes of immuno-chemistry than of any other chemical reaction. This is so much the more fortunate as, on studying their effects, and especially the measurement of their strength, one is obliged to learn their rate of reaction very exactly and to study the factors which bear upon it. Many are the mistakes which have been made in the course of time in this domain, when it has been incorrectly believed that one could determine a change in the strength of the reacting substances, where in reality there was only a change in the *velocity of reaction.*

In order to be able to work quantitatively with some certainty with the substances with which we have to do in the science of immunity, it is therefore necessary to try to interpret the laws

of the reactions of antigens and antibodies, separately and on each other. Work of this kind has been continued systematically at the Serum Institute in Copenhagen ever since the beginning of the century, and among my collaborators there is especially *Walbum*. It is obvious that the problem is exceedingly difficult, having regard to the fact that one is working with chemically unknown substances in a mixture of a lot of other substances which, for the greater part, are also unknown, and whose influence upon the course of the reaction one does not know and can only gradually conjecture.

When one finds a regular course in a process, it is necessary first of all to examine whether this can be attributed to one of the *simple laws* which apply in physical chemistry. If calculations show that, for instance, a reaction can be expressed by the same formula which applies to monomolecular reaction, the most simple course of reaction, there is every probability that we really have monomolecular reaction before us. If, on the other hand, a phenomenon is observed, for example, a weakening of coli-agglutinin which by heating sometimes progresses like a monomolecular reaction, but sometimes differently so that the reaction cannot be classified under the reactions known in physical chemistry, it is very probable that the reaction in reality is inclined to progress according to the monomolecular law, but that in the liquid (in this case the coli-agglutininous serum) there are substances which are affecting the course of the reaction, or that perhaps during the reaction itself substances are being formed which affect the reaction curve. This latter we are only too familiar with from the course of reaction of known chemical substances, and, under these circumstances, we are able to express this more complicated course of reaction in a formula.

But in our investigations we have not striven for this. We have principally tried to find the processes which have advanced according to simple laws; it was our aim in the first place to show that these applied here as in all other chemistry. Of course, it is possible to put up formulas for the other processes with vary-

ing courses, but hitherto this has seemed to us to be valueless as long as these formulas were only empirical and did not coincide with others which express a reaction of known nature. Gradually as we have published our material we have therefore given these series of experiments without calculations.

Our investigations may be divided into three large groups, which comprise: (1) the laws of the action of antigens and antibodies on their medium, (2) the laws of the destruction of antigens and antibodies, and (3) the laws of the interaction of antigens and antibodies.

Thus the question is one of the closer study of these reactions and of a series of factors which have an influence upon the velocity of reaction, principally the hydrogen-ion concentration and the temperature.

Our experiments comprise substances of very different natures: enzymes, such as trypsin, pepsin, rennet, pyocyaneus-trypsin, fibrin-ferment; lysins, such as vibriolysin, tetanolysin, streptolysin, epeiralysin and epeira-toxin, the hæmolytic and toxic substances found in the poison of epeira diadema, the cross-spider, bee poison, wasp poison; toxins, such as diphtheria toxin, tetanus toxin, botulinus toxin, complement, amboceptor, different agglutinins; antitoxins, such as diphtheria and tetanus antitoxin, and several others.

I will not now commence upon a dissertation of our results with all these substances; it would only tire you; but I will limit myself to giving you some examples of the influence of *temperature* on these processes.

It is a well known fact that temperature has a considerable effect upon most chemical processes; in general, reactions proceed very much more rapidly at higher than at lower temperatures. This also applies in general for biological processes; from the care with which the warm-blooded organism seeks to keep the temperature constant one can estimate the importance of fluctuations of temperature.

I will first show you an example of a toxin destruction from

a series of experiments I have made with *Famulener.* It was made with *vibriolysin,* the hæmolytic substance which is found in cultures of certain cholera-like vibrions. One places a jena flask with vibriolysin in a water bath with a very exactly adjusted temperature. That the exactness of the temperature must be exceedingly great is clear from the following. Then, at certain intervals, samples are taken of the liquid, which are at once cooled down to about 0°, and the hæmolytic capacity of which is then measured by the method described by me. You will see the result from the following table. The experiment was made at 48.15.°

TABLE 1

*Destruction of Vibriolysin at 48.15°*

| t (min.) | q (obs.) | q (calc.) | t (min.) | q (obs.) | q (calc.) |
|---|---|---|---|---|---|
| 0 | 100 | 100 | 40 | 12.1 | 12.6 |
| 10 | 53 | 59.6 | 50 | 7.4 | 7.5 |
| 20 | 35 | 35.7 | 60 | 5.0 | 4.5 |
| 30 | 22 | 21.1 | 70 | 2.7 | 2.7 |

K = 0.0225

In this table you will see first the time (t) when the samples are taken; then under q the hæmolytic power of the toxicity observed, expressed by calling it 100 at the commencement of the experiments and then declining towards O. As regards the progress of time of this decomposition, it behaves precisely as an ordinary monomolecular reaction, the formula of which is:

$$-\frac{dq}{dt} = Kq$$

where q is the toxicity at the time t and where K is a constant which signifies the velocity of the reaction.

In the last column there are the values thus calculated. You will see that the agreement is satisfactory, especially when it is taken into consideration how great the errors of experiment in

these determinations are. The reaction constant was found to be = 0.0225.

Similar experiments made at a number of other temperatures give a conforming result; they could all be expressed by the same monomolecular formula. But it appeared at the same time that the velocity at which the weakening proceeded changed very much. Whilst the toxicity at 49.975° after only four minutes was reduced to half, at 48.15° it takes ten minutes and at the three degrees lower temperature, 45.15°, about 80 minutes.

For each of these reactions one can calculate the constants of the reaction (K). If one draws up these reaction velocities, one gets the following table:

TABLE 2

*Relation between Temperature and Rate of Destruction of Vibriolysin (k)*

| Temp. | K (obs.) | K (calc.) | Temp. | K (obs.) | K (calc.) |
|---|---|---|---|---|---|
| 49.975° | 0.0778 | 0.0741 | 47.035° | 0.0134 | 0.0128 |
| 49.75° | 0.066 | 0.0656 | 46.40° | 0.0079 | 0.00811 |
| 49.00° | 0.0391 | 0.041 | 45.97° | 0.00625 | 0.00618 |
| 48.15° | 0.0225 | 0.024 | 45.65° | 0.00575 | 0.00505 |
| 48.08° | 0.0236 | 0.023 | 45.145° | 0.0036 | 0.0036 |

K = 128570

For the dependence of chemical processes upon temperature the van't Hoff-Arrhenius formula applies, as you know:

$$\frac{K_1}{K_2} = e^{\frac{\mu}{2}\left(\frac{T_1 - T_2}{T_1 T_2}\right)}$$

when $T_1$ and $T_2$ are the temperatures reckoned from the absolute zero. $K_1$ is the velocity of reaction at the temperature $T_1$ and $K_2$ that at $T_2$, $\mu$ is a constant. The greater $\mu$ is, the more rapidly the velocity of reaction increases with the temperature.

If we now try how these reaction velocities conform to the formula, we get, as Table 2 shows, extremely close agreement.

In the first row the temperature is shown; in the second the

observed velocity of reaction (K); in the third the agreements calculated according to the formula are, as you can see, particularly good. The constant $\mu$ is 128570, *i.e.*, very high.

It appears, therefore, that destruction, theoretically speaking, proceeds extremely slowly at lower temperature, at higher temperatures so quickly that measurement is impossible.

A remarkable thing is the very great influence which temperature has here in the velocity of reaction; it increases almost 100 per cent. for every degree the temperature rises; this is much more than is the case with ordinary chemical processes. If one considers the classic examples, the temperature influence upon the inversion of cane sugar is such that the velocity of reaction is doubled when the temperature rises 5° (corresponding to $\mu$–25640) and at the saponification of æthyl acetate a temperature increase of about 10° is required ($\mu$–11160 col. per gr. molecule).

The lessons from the example I have chosen to show you, vibriolysin, are repeated in a large number of other examined substances, tetanolysin, epeiralysin, epeira-toxin, in enzymes like trypsin, pepsin (although not in all cases), pyocyaneus-trypsin, rennet, fibrin ferment, and many others, which all follow the same laws of destruction.

It is not always, however, that an *increase* of effect is consequent upon a rise of temperature. On experimenting with a large number of hæmolytic substances, such as acids, alkalies, snake poisons, etc., Madsen, Noguchi and Walbum found that this was the case, as a rule, but that there were exceptions. Thus it was found that the hæmolytic power of tetanolysin had an optimum at 31°, staphylolysin an optimum between 20° and 30°, and with the poison of the water mocassin, ancistrodon piscivorus, there was the peculiarity that the effect gradually increased with falling temperature. Walbum has also found an optimum for epeiralysin; after ten minutes' observation it lies at about 32°, but gradually moves downwards so that after five hours' observation it is at 16°; special experiments showed that it was not a question of destruction of the lysin at these temperatures.

Sometimes *cooling* has a peculiar effect upon the hæmolytic substances.

It would at first be thought that the more the temperature was lowered, the better would these substances be preserved. If we return to the Arrhenius formula I have just referred to, you will remember that the velocity of destruction on the one hand rose very rapidly, but on the other hand it fell very quickly when the temperature was lowered. One can calculate from the formula how great the weakening should theoretically be at lower temperatures.

TABLE 3

*Rate of Destruction of Vibriolysin at Different Temperatures Calculated After Table 2*

| Temp. | K | Temp. | K |
|---|---|---|---|
| 54° | 0.87 | 46° | 0.00628 |
| 49° | 0.041 | 45° | 0.00334 |
| 48° | 0.022 | 37° | 0.000018 |
| 47° | 0.0118 | 18° | 0.0000000000238 |

A reduction from 100 to 1 per cent., which at 49° proceeds in one hour, demands at 54° only a couple of minutes; but at 37° it would require five to six months, and at 18° several hundred thousand years.

Now we know very well that this is not the case. These substances weaken perceptibly, although slowly both at 37° and at 18° room temperature. Thus other laws obviously apply here.

Thus it appears from some experiments recently made by *Reymann* in our institute that the weakening of tetanolysin at lower temperatures proceeds much more quickly. At 37° the *tetanolysin* has, even after 24 hours, lost a great part of its strength; the same thing, although to lesser degree, was observed at 18° and 8° . Even at Zero and at –10° there commences, after a slight increase, a slow decline. On the other hand, if we go down to very low temperatures, –70° and –180°, there appears in the course of some hours a perceptible increase of lysin power

which thereafter remains fairly constant for a long time. *Walbum* had already found something similar with *bee poison, wasp poison* and *cobra lysin;* on cooling down to –180° (with liquid air) there was a very perceptible increase of their strength.

On the other hand, there are poisons where the opposite is the case; if one cools *epeiralysin* to –180°, it at once *loses up to 50 per cent.* of its hæmolytic power. It is of interest to bear this in mind, for, as a rule, one preserves one's substances at a low temperature, this being especially true of standard sera and standard toxin, which are often kept at temperatures round about –10°.

### DIPHTHERIA TOXIN

If we turn to the consideration of one of our most important toxins, the *diphtheria toxin,* our findings are more complicated. In spite of the fact that it was with this toxin Ehrlich made his famous investigations into the measurement of antidiphtheritic serum, the laws for its deterioration have hitherto not been thoroughly studied.

Table 4 represents the weakening of this toxin, preserved at a temperature of 2°–4°.

TABLE 4

*Decrease of Toxicity of Diphtheria Toxin at 2°-4°*

I

| Date | Toxicity (obs.) | Toxicity (calc.) |
|---|---|---|
| 28/10 97 | 143 | 143 |
| 1/1 98 | 104 | 115 |
| 11/8 — | 89 | 84 |
| 27/8 — | 69 | 69 |
| 13/8 — | 67 | 67 |
| 17/10 — | 50 | 63 |

II

| Date | Toxicity (obs.) | Toxicity (calc.) |
|---|---|---|
| 1/11 1901 | 476 | 476 |
| 15/1 02 | 455 | 435 |
| 1/2 — | 417 | 400 |
| 19/6 — | 286 | 345 |
| 1/11 — | 286 | 294 |
| 1/7 03 | 185 | 233 |
| 15/9 — | 213 | 222 |
| 19/2 04 | 213 | 208 |

This slow weakening proceeds rather irregularly, but may be approximately expressed by the formula which is called the

qt law, where the product of the time t and the quantity used is constant.

The following table:

TABLE 5

*Weakening of Diphtheria Toxin at Different Temperatures (Walbum)*

| t days | $P_H$=7.40 | | | | $P_H$=8.35 | | | |
|---|---|---|---|---|---|---|---|---|
| | 25° | 30° | 35° | 40° | 25° | 30° | 35° | 40° |
| 0 | 143 | 143 | 143 | 143 | 143 | 143 | 143 | 143 |
| 2 | 133 | 125 | 125 | 100 | 111 | 100 | 91 | 71 |
| 5 | 118 | 111 | 100 | 91 | 100 | 91 | 91 | 50 |
| 9 | 118 | 111 | 91 | 83 | 100 | 83 | 71 | 37 |
| 18 | 111 | 100 | 91 | 71 | 91 | 83 | 63 | 31 |
| 30 | 111 | 100 | 83 | 67 | 91 | 83 | 59 | 25 |

shows the destruction of diphtheria toxin at higher temperatures, 25°, 30°, 35° and 40°, and at two different hydrogen-ion concentrations, 7.40 and 8.35. The course of this reaction does not follow any simple law, and therefore I shall not go further into

TABLE 6

*Destruction of Diphtheria Toxin at Different Temperatures*

| 60° | | | 57° (I) | | | 57° (II) | | | 54° | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| t (min.) | q (obs.) | q (calc.) | t (min.) | q (obs.) | q (calc.) | t (min.) | q (obs.) | q (calc.) | t (min.) | q (obs.) | q (calc.) |
| 0 | 47.6 | 47.6 | 0 | 105 | 105 | 0 | 143 | 143 | 0 | 167 | 167 |
| 2 | 15.4 | 18.2 | 2 | 37 | 37 | 10 | 31 | 10.5 | 2 | 66.7 | 66.7 |
| 5 | 9.1 | 10.5 | 5 | 18.2 | 18.2 | 30 | 4.35 | 3.92 | 5 | 25 | 40 |
| 10 | 4.8 | 6.2 | 10 | 11.1 | 10.5 | 60 | 2.0 | 2.0 | 10 | 16.7 | 22.2 |
| 15 | 4.3 | 4.3 | 20 | 5.56 | 5.56 | 90 | 1.43 | 1.33 | 20 | 11.1 | 12.2 |
| 20 | 3.4 | 3.4 | 30 | 4.17 | 3.77 | 120 | 1.0 | 1.0 | 30 | 8.3 | 8.3 |
| 25 | 3.1 | 2.8 | 40 | 2.86 | 2.86 | 180 | 0.53 | 0.67 | 40 | 6.7 | 6.7 |
| 35 | 2.2 | 2.5 | 60 | 2.13 | 1.92 | | | | 60 | 4.0 | 4.3 |
| 40 | 2.0 | 1.75 | 80 | 1.43 | 1.43 | | | | 90 | 3.3 | 3.0 |
| 50 | 1.43 | 1.43 | 100 | 1.25 | 1.15 | | | | 120 | 2.2 | 2.2 |
| 60 | 1.33 | 1.19 | 120 | 1.03 | 0.96 | | | | 180 | 1.67 | 1.52 |
| 70 | 1.25 | 1.02 | 150 | 0.77 | 0.77 | | | | 240 | 1.43 | 1.14 |
| 80 | 1.0 | 0.89 | 180 | 0.63 | 0.65 | | | | 300 | 0.7 | 0.9 |
| 100 | 0.7 | 0.7 | 240 | 0.5 | 0.48 | | | | 360 | 0.56 | .75 |
| 120 | 0.59 | 0.6 | | | | | | | 480 | 0.59 | 0.53 |
| K=0.0137 | | | K=0.0086 | | | K=0.0083 | | | K=0.00368 | | |

$\mu$=47800

it, but shall simply draw your attention to two things; firstly, that the hydrogen-ion concentration, of course, affects the velocity of reaction; secondly, that the temperature will, no doubt, increase that velocity, but not to any particularly marked degree, and that their influence can by no means be compared with what we saw previously as far as vibriolysin and tetanolysin are concerned.

At higher temperatures the reaction proceeds much more rapidly, as Table 6 shows:

The reaction was measured at three temperatures, 60°, 57° (twice) and 54°. This agrees, with very close approximation, with the qt law, as the table shows (q calc.). Dependence upon temperature is much greater than in the previous experiments at lower temperature, the velocity of reaction being almost doubled at a temperature rising from 3°. In this experiment the temperature coefficient is 47800.

### WEAKENING OF DIPHTHERIA ANTITOXIN

Thorough knowledge of the laws of the weakening of diphtheria antitoxin under different conditions is not alone of interest theoretically, but is a matter of great practical importance as well.

We have long been cognizant of the fact that when diphtheria serum is kept for some time at ordinary room temperature or lower, it gradually loses its antitoxic potency.

At higher temperature, especially when heated to about 70°, the potency of the antitoxin is very quickly diminished. This phenomenon must be borne in mind when pasteurizing diphtheria serum, a method of sterilization used at many institutes, but first and foremost at l'Institut Pasteur. The matter is of even greater interest in using the newest methods of concentration, especially the Homer method, in which the serum is heated to about 61° in order to change the normal precipitation boundaries.

In spite of the practical importance of the problem, as yet no rational investigation of the weakening of diphtheria antitoxin at higher temperature has been made, and on that account we have made the question the object of several experiments.

A series of preliminary investigations showed that when the serum was used undiluted, the temperature at which antitoxin destruction was practically measurable lay above the coagulation temperature. In order to draw samples of the heated serum, it was necessary, then, to prevent coagulation. For this a dilution, in the proportion 1 part serum to 3 parts 0.9 per cent. sodium chloride solution, was found suitable.

The electrometric test showed the hydrogen-ion concentration of this serum dilution to be $P_H - 7.61$. As is known, hydrogen-ion concentration diminishes when the protein substances are coagulated (S. P. L. Sörensen). Here, too, diminution was observed, for $P_H = 8.09$ after the serum dilution had been heated 20 minutes to 72° C. The liquid was then strongly opalescent and could not bear more heat without becoming gelatinous.

The experiments were made at 5 different temperatures: 74.1°, 72.05°, 70.0°, 67.95°, 65.75°. A flask of Jena glass with the serum dilution was placed in a water bath, and as soon as the dilution had attained the same temperature as the bath, sampling began. Each sample was immediately reduced in temperature by being placed in ice-water. The antitoxin content of the samples was measured by the Ehrlich method. The antitoxin strengths of the undiluted serum was 320 antitoxic units per $cm^3$, in the diluted 80 A.U. per $cm^3$.

The details may be seen in Table 7; the first column shows the time (t) in minutes, the second the values of the antitoxin content of the serum observed (A. U. obs.).

At 74.1° the initial power observed is only 50 A. U., which indicates that even heating to the temperature of the experiment weakens the antitoxin considerably. In an hour's time by far the larger part of the antitoxin is destroyed, there being only 2 A. U. per $cm^3$ left.

At lower temperatures weakening occurs much more slowly, at 65.75°, after 6 hours, it is only reduced to 30 per cent. of the original strength. The course of this reaction can satisfactorily be calculated as a bimolecular process, as it is appearing in the third column in the table (A. U. calc.). If we investigate the relation between the temperatures and the reaction speed we find

TABLE 7

*Destruction of Diphtheria Antitoxin at Different Temperatures*

| 74.1° | | | 72.05° | | | 70.0° | | | 67.95° | | | 65.75° | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| t min.) | A. U. (obs.) | A. U. (calc.) | t (min.) | A. U. (obs.) | A. U. (calc.) | t (min.) | A. U. (obs.) | A. U. (calc.) | t (min.) | A. U. (obs.) | A. U. (calc.) | t (min.) | A. U. (obs.) | A. U. (calc.) |
| 0 | 50 | 50 | 0 | 70 | 70 | 0 | 75 | 75 | 0 | [80] | 68 | 0 | 80 | 80 |
| 4 | 25 | 25 | 7 | 35 | 37.7 | 10 | 45 | 51.3 | 10 | 60 | 60 | 30 | 65 | 64.9 |
| 7 | 20 | 19.2 | 15 | 25 | 25 | 30 | 25 | 31 | 30 | 45 | 48 | 60 | 55 | 54.9 |
| 15 | 12 | 11.4 | 20 | 20 | 20.4 | 60 | 20 | 19.4 | 60 | 35 | 37 | 120 | 40 | 43.5 |
| 20 | 10 | 9.1 | 30 | 15 | 15.3 | 100 | 15 | 13 | 90 | 30 | 30 | 180 | 35 | 35 |
| 40 | 5 | .5 | 50 | 10 | 10 | 150 | 10 | 9.2 | 120 | 25 | 25 | 360 | 25 | 22.5 |
| 60 | 2 | 3.4 | 80 | 7 | 6.7 | 180 | 7 | 7.8 | 180 | 20 | 19 | | | |
| | | | 110 | 5 | 5 | | | | 240 | 15 | 15.4 | | | |
| K = 0.0045 | | | K = 0.0017 | | | K = 0.00064 | | | K = 0.00022 | | | K = 0.000089 | | |

that the reaction speed diminishes greatly with decreasing temperature; for every 2° it is diminished about 2.6 times, so that at 65.75° it is about 51 times lower than at 74.1°; in other words the weakening, which occurs in an hour at a temperature of 74.1°, requires 51 hours at a temperature of 65.75°.

TABLE 8

*Synopsis of Table 6*

| Temp. | K (obs.) | K (calc.) |
|---|---|---|
| 74.10° | 0.0045 | 0.0045 |
| 72.05° | 0.0017 | 0.00176 |
| 70.00° | 0.00064 | 0.000648 |
| 67.95° | 0.00022 | 0.000267 |
| 65.75° | 0.000089 | 0.000088 |
| | $\mu - 110800$ | |

In Table 8, under column K (calc.), in which appear the values reckoned for K, we note that within the temperature intervals used there is excellent agreement between the values observed and computed. The temperature constant $\mu$ is 110800, that is to say, quite high; this corresponds to what was found by *Madsen* and *Streng* to be the case with coli-agglutinin from goats and rabbits in which the size of $\mu$ varied between 93400 and 109600.

It is generally acknowledged that temperatures in the vicinity of 70° are dangerous for the antitoxin; however, this is not the case with temperatures between 50° and 60°. These are, indeed especially interesting as 56° is the degree of heat usually used for pasteurizing diphtheria serum in part to sterilize and in part to rid the serum of some of its toxic qualities.

If we examine Table 7 we note that at 65.75° a weakening of ca 20 per cent.—the highest permissible by manipulating to sterilize or concentrate serum—is observed after one-half an hour has passed. Extrapolarizing with the above material as point of departure we find that the 20 per cent. weakening at 60° requires 8½ hours and at 56° about 32½ hours. The routine heating to 56° for ½ hour each day for 3 successive days seems to proceed without a too great loss of antitoxin, which would be the case if the temperature was increased. The fact that $\mu$ is so high

accounts for the fact that even a slight rise in the temperature is followed by a considerable increase of the rate of destruction.

In these considerations we must remember that the experiments were made with serum dilutions and that with pure serum the conditions may be quite different.

Changes in the hydrogen-ion concentration will presumably also affect the reaction speed.

Just as interesting as the rapid weakening of the diphtheria antitoxin at high temperatures is the diminution in potency to which it is subjected when kept for a long time at room temperature or in cold storage. In the early days of serum therapy, there was a constant demand for "fresh serum" which decreased as the realization became general that the decrease in antitoxic potency was comparatively small under storage, while on the other hand "storing" serum seemed to diminish serum attacks.

Germany's government control takes this decrease into consideration and requires that the bottles of diphtheria antitoxin sent out be tested 6 and 24 months after delivery. If a decrease in antitoxin potency of more than 10 per cent. is observed, the preparation is recalled. According to *Marx*,[2] about 3 per cent. of the bottles are recalled.

Many scientists have published data which contribute to our knowledge of the decrease in antitoxin potency under prolonged storage. Among these may be mentioned Marx, Müller, Layson, Kinyon and Hitchens. However, the investigations are not sufficiently detailed to warrant our drawing conclusions in regard to the reaction speed.

The long series of investigations made by *John F. Anderson*,[3] on the other hand, permit us to do so.

For several years he has observed the decrease in potency of the antitoxin at 3 degrees of heat, at room temperature, which he places at figures varying betwen 70° F. in winter and 95°–100°

[2] Marx: Mitteilungen aus der prüfungstechnischen Praxis. Festskrift for Robert Koch.

[3] John F. Anderson: The influence of age and temperature on the potency of diphtheria antitoxin. Hygienic Laboratory Bulletin, Nr. 66, 1910.

F. in the summer; at 15° C. and at 5° C. The sera used by *Anderson* were of very different antidiphtheric potencies, from 240 to 1500 A. U. per cm.[3]

TABLE 9

*Weakening of Diphtheria Antitoxin at Lower Temperatures (J. F. Anderson)*

| C 25° | | | | 15° | | | | 5° | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| t (days) | A. U. (obs.) | A. U. (mon.) | (calc.) (Cim.) | t (days) | A. U. (obs.) | A. U. (mon.) | (calc.) (Cim.) | t (days) | A. U. (obs.) | A. U. (mon.) | (calc.) (Cim.) |
| 0 | 660 | 660 | 660 | 0 | 660 | 660 | 660 | 0 | 660 | 660 | 660 |
| 183 | 580 | 557 | 575 | 183 | 600 | 600 | 603 | 183 | 620 | 625 | 625 |
| 366 | 500 | 481 | 500 | 366 | 580 | 554 | 564 | 366 | 620 | 595 | 600 |
| 544 | 440 | 424 | 444 | 544 | 500 | 514 | 520 | 544 | 580 | 568 | 570 |
| 742 | 340 | 376 | 385 | 730 | 500 | 478 | 484 | 730 | 540 | 543 | 545 |
| 1089 | 300 | 313 | 300 | 1089 | 420 | 420 | 420 | 1089 | 500 | 500 | 500 |
| K = 0.00000155 | | | | K = 0.00000079 | | | | K = 0.000000445 | | | |

In spite of the inequalities in the experimental conditions, the decrease seemed to advance quite regularly. In Table 9 we have presented some of Anderson's data so that they may be compared with our own experiments in heating. They are, however, not carried out far enough—at the most only to a weakening of 50 per cent.—to enable us to say anything about the course of the process; the first part may be expressed with practically the same exactitude as a bimolecular or monomolecular reaction (see the table).

It is therefore impossible to determine whether the spontaneous weakening at a low temperature is a process of the same species as that which occurs under heating to about 70°.

On the other hand, it is very evident that temperature is otherwise a quite important factor for the reaction speed. In

TABLE 10

*Synopsis of Table 9*

| Temp. | K (obs.) | K (calc.) |
|---|---|---|
| 25° | 0.00000155 | 0.00000155 |
| 15° | 0.00000079 | 0.00000079 |
| 5° | 0.000000445 | 0.000000445 |

$\mu = 11100$

Table 10 are listed the reaction constants from Table 9 for comparison—and the varying conception "room temperature" (see above) is placed at 25° C. We note that when the temperature increases 10°, the reaction speed nearly doubles, which, according to the van't Hoff-Arrhenius equation corresponds to a value of $\mu$ of about 11100 cal.

The effect of temperature on reaction speed under these conditions then is only 1/10 of what it is at a temperature of 70° ($\mu = 110800$), and these two processes may then not be considered identical. If the destruction speed should decrease in the same proportion as it does from 74.10° to 65.75°, a measurable weakening at 20° would require millions of years.

It is evident, then, that at high temperature other factors, too, are involved.

### TOXIN-ANTITOXIN

In the foregoing I have treated the effect of temperature upon *diphtheria toxin* and *diphtheria antitoxin* separately. Now I would briefly touch upon the question of the law governing the effect of these two substances *upon each other.*

This brings us to the question of the law regarding the neutralization of toxin and antitoxin.

Perhaps some of you remember that Arrhenius and I once showed that the curve of neutralization should not, as maintained by Ehrlich, be expressed like a staircase, but that in reality it proceeds as a quite even curve, and furthermore that we expressed the opinion that the curve of neutralization was very similar to that found between slightly dissociated substances and that the combination between the two substances was of a slow nature.

Without going further into this complicated question, which would take us too far, I would remind you that later investigations have proved that we were right on this point, as it has been possible, at any rate to a great extent, to cause the dissociation of the toxin-antitoxin complex.

It will hardly be possible to go into a closer study of these complicated questions before one is able to work with both com-

ponents in a pure, or considerably purer state than they are in at present. Recent years have, however, taken us well along the road of progress, since authors like Ramon have shown that the neutralization between diphtheria toxin and diphtheria antitoxin can be pursued in vitro.

If to a number of test tubes, each containing the same quantity of strong diphtheria toxin, we add gradually smaller quantities of antitoxin, a precipitation will be observed at a place which begins just where the neutral mixture of toxin and antitoxin is, and which declines to both sides with greater or smaller additions of antitoxin.

The maximum of the precipitation corresponds on the whole to the point of neutralization we find in animal investigations, and this method has therefore proved to be a particularly valuable help in a practical titrating of diphtheria serum. It also seems capable of giving us valuable information as to the relations between toxin and antitoxin, and we have subjected the reaction to an investigation in several respects. In connection with the subject of my lecture I would describe some investigations into the influence of temperature upon this precipitation reaction.

TABLE 11

*Relation between Temperatures and Ramon's Reaction (Madsen and Schmir)*

| Temp. | Time in hours | | Velocity | |
|---|---|---|---|---|
| | (obs.) | (calc.) | (obs.) | (calc.) |
| 62° | 4 | | | |
| 56° | 1 | 1 | 100 | 100 |
| 45° | 2 | 2.07 | 50 | 48.4 |
| 37° | 3 | 3.64 | 33 | 27.5 |
| 30° | 7 | 6.02 | 14.3 | 16.6 |
| 20° | 19 | 13.2 | 5.3 | 7.6 |
| 12° | 25 | 24.1 | 4 | 4.15 |
| 6° | 40 | 40 | 2.5 | 2.5 |
| 3° | 45 | 56.2 | 2.2 | 1.8 |
| 0° | 62 | 68.5 | 1.6 | 1.46 |

$\mu = 13540$

From Table 11 it is obvious that the optimum is near 56°, at which temperature the reaction is finished after 1 hour, at

higher temperature the process takes longer time, *e.g.*, at 62° 4 hours. The experiment, shown in Table 12 demonstrates that in this case there is no difference between 55° and 65°; at 70° the reaction goes slower.

TABLE 12

*Ramon's Reaction and Temperature*

| Temp. | Time in hours | | Temp. | Time in hours | |
|---|---|---|---|---|---|
| | (obs.) | (calc.) | | (obs.) | (calc.) |
| 70° | 1.5 | | 30° | 2.50 | 2.6 |
| 65° | 0.67 | | 25° | 4.33 | 3.63 |
| 60° | 0.67 | | 21° | 5.25 | 4.7 |
| 55° | 0.67 | 0.60 | 15° | 7.16 | 7.16 |
| 50° | 0.83 | 0.79 | 12° | 8.33 | 8.8 |
| 45° | 1.0 | 1.06 | 9° | 12 | 11 |
| 40° | 1.42 | 1.41 | 7° | 14 | 12.6 |
| 35° | 1.66 | 1.9 | 0° | 17 | 21.1 |

$\mu = 11600$

Investigation into the relation of velocity of reaction to temperature shows that it can be expressed with fair exactness by the van't Hoff-Arrhenius law, if, for example, we call the course of reaction at 56° (1 hour) 100, at 45° (2 hours), 50, and so on. The table shows that the agreement is satisfactory. The temperature coefficient, 13540, is low, compared with our knowledge of the reactions previously mentioned. Table 12 is calculated after the same principle and shows also a fairly good agreement except at 0°, where obviously the errors of experiment are rather great. The temperature coefficient 11600 is about the same in the previous experiment. It is in the same order of size as the saponification of an æthyl acetate (11160). This means, then, that with a temperature increase of 10° we get a doubling of the velocity. This figure also corresponds to what is found in various agglutination processes, and probably gives an idea of the coefficient of temperature for neutralization of diphtheria toxin by diphtheria antitoxin.

The difficulty in the measurement of the influence of temperature, by the help of animals, lies, for one thing, in the fact that even if one can keep the mixture of toxin and antitoxin at vari-

ous low temperatures, they will immediately be heated up to 37° when injected into a warm-blooded animal, and this is something that hitherto has not been avoidable by the Ehrlich method.

### COMPLEMENT AND AMBOCEPTOR

The hæmolytic system plays such a predominant rôle that a thorough study of its physical chemistry was necessary.

A long time ago Famulener and Madsen found that a natural hæmolytic system, goat serum, which has a strong hæmolytic action upon rabbit red blood-cells, was exceedingly susceptible to heating, as appears from Table 13.

TABLE 13

*Hemolytic Goat Serum*

| 53° | | | 52.5° | | | 52° | | |
|---|---|---|---|---|---|---|---|---|
| t (min.) | q (obs.) | q (calc.) | t (min.) | q (obs.) | q (calc.) | t (min.) | q (obs.) | q (calc.) |
| 0 | 100 | 100 | 0 | 100 | 100 | 0 | 100 | 100 |
| 2.5 | 58 | 58.2 | 2.5 | 64 | 70.8 | 5 | 63 | 65 |
| 5 | 44 | 33.4 | 5 | 49 | 50.1 | 10 | 39 | 42.4 |
| 7.5 | 18 | 19.3 | 7.5 | 37 | 35.5 | 15 | 27.5 | 27.5 |
| | | | 10 | 25 | 25.1 | 20 | 18 | 17.8 |
| | | | 12.5 | 20 | 17.6 | 25 | 13 | 11.6 |
| K = 0.0953 | | | K = 0.06 | | | K = 0.0374 | | |

| 51.5° | | | 51° | | |
|---|---|---|---|---|---|
| t (min.) | q (obs.) | q (calc.) | t (min.) | q (obs.) | q (calc.) |
| 0 | 100 | 100 | 0 | 100 | |
| 5 | 85 | 74.8 | 5 | 74 | 73.4 |
| 10 | 62 | 56.2 | 10 | 59 | 62.5 |
| 15 | 37 | 42.2 | 15 | 49 | 53.3 |
| 20 | 29 | 31.6 | 20 | 45 | 45.4 |
| 25 | 26 | 23.7 | 25 | 40 | 38.7 |
| 30 | 17 | 17.8 | 30 | 33 | 33 |
| | | | 35 | 28 | 28.1 |
| | | | 40 | 25 | 24 |
| K = 0.025 | | | K = 0.0139 | | |

The synopsis, *viz.*,

TABLE 14

*Synopsis of Table 13*

| Temp. | K (obs.) | K (calc.) |
|---|---|---|
| 53° | 0.0953 | 0.0955 |
| 52.5° | 0.06 | 0.0598 |
| 52° | 0.0375 | 0.0375 |
| 51.5° | 0.025 | 0.0235 |
| 51° | 0.0139 | 0.0145 |

$\mu = 198500$

makes it clear how enormous the influence of the temperature is, $\mu$ being = 198500; this corresponds to an increase in the rate of destruction of 2.6 times for a rise of temperature of 1 degree, the greatest figure of this kind hitherto observed.

Of especial interest is the study of the *complement*. The course of its destruction has been proved to follow in most cases the monomolecular formula and the relation between the rate of reaction and the temperature followed the usual temperature law. We have examined complements from different animals, such as swine, horse and guinea-pig, and all followed the same law. Table 15 represents a swine-complement; Table 16 a complement from guinea-pig.

TABLE 15

*Swine Complement. Heating at Different Temperatures*

| 56.012° | | | 55.11° | | | 54.012° | | | 53.175° | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| t (min.) | q (obs.) | q (calc.) | t (min.) | q (obs.) | q (calc.) | t (min.) | q (obs.) | q (calc.) | t (min.) | q (obs.) | q (calc.) |
| 0 | 10 | 10 | 0 | 10 | 10 | 0 | 10 | 10 | 0 | 10 | 10 |
| 1 | 3.7 | 4.0 | 1 | 5.0 | 5.4 | 3 | 2.66 | 3.16 | 3 | 5.0 | 4.65 |
| 2 | 1.54 | 1.54 | 2 | 3.3 | 2.9 | 6 | 1.23 | 1.0 | 6 | 2.0 | 2.15 |
| 3 | 1.0 | 0.62 | 3 | 1.54 | 1.54 | | | | 9 | 1.0 | 1.0 |
| | | | | | | | | | 12 | 0.5 | 0.47 |
| K = 0.403 | | | K = 0.271 | | | K = 0.167 | | | K = 0.111 | | |

TABLE 15—*Continued.*

| 52.012° | | | 51.06° | | | 50.012 | | |
|---|---|---|---|---|---|---|---|---|
| t (min.) | q (obs.) | q (calc.) | t (min.) | q (obs.) | q (calc.) | t (min.) | q (obs.) | q (calc.) |
| 0 | 10 | 10 | 0 | 10 | 10 | 0 | 10 | 10 |
| 4 | 6.0 | 5.0 | 4 | 6.25 | 6.25 | 5 | 7.7 | 7.1 |
| 8 | 3.0 | 2.55 | 8 | 3.85 | 3.85 | 10 | 5.0 | 5.05 |
| 12 | 1.0 | 1.3 | 12 | 2.0 | 2.4 | 15 | 3.85 | 3.6 |
| 16 | 0.5 | 0.66 | 16 | 1.43 | 1.47 | 20 | 2.5 | 2.55 |
| | | | 20 | 1.0 | 0.92 | 30 | 1.25 | 1.3 |
| | | | | | | 40 | 0.63 | 0.65 |
| | | | | | | 50 | (0.5 | 0.34) |
| K = 0.074 | | | K = 0.05 | | | K = 0.0295 | | |

TABLE 16.

*Guinea-Pigs Complement. Heating at Different Temperatures*

| 58.02° | | 57.13° | | 56.02° | | 55.03° | | | 54.02° | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| t (min.) | q (obs.) | t (min.) | q (obs.) | t (min.) | q (obs.) | t (min.) | q (obs.) | q (calc.) | t (min.) | q (obs.) | q (calc.) |
| 0 | 62.5 | 0 | 62.5 | 0 | 125 | 0 | 125 | 125 | 0 | 154 | 154 |
| 1 | 10 | 1 | 15.4 | 2 | 15.4 | 2 | 33.3 | 35 | 3 | 50 | 59 |
| | | | | | | 4 | 10 | 10 | 6 | 20 | 23 |
| | | | | | | | | | 9 | 10 | 9 |
| K = 0.8 | | K = 0.61 | | K = 0.455 | | K = 0.27 | | | K = 0.14 | | |

| 53.13° | | | 52.03° | | | 51.25° | | | 50.13° | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| t (min.) | q (obs.) | q (calc.) | t (min.) | q (obs.) | q (calc.) | t (min.) | q (obs.) | q (calc.) | t (min.) | q (obs.) | q (calc.) |
| 0 | 154 | 154 | 0 | 200 | 200 | 0 | 200 | 180 | 0 | 200 | 180 |
| 3 | 62.5 | 62 | 4 | 125 | 115 | 4 | 125 | 122 | 5 | 154 | 142 |
| 6 | 25.0 | 25.0 | 8 | 62.5 | 66 | 8 | 77 | 83 | 10 | 100 | 111 |
| 9 | 12.5 | 10 | 12 | 50.0 | 38 | 12 | 56 | 56 | 15 | 77 | 87 |
| | | | 16 | 22.2 | 22 | 16 | 40 | 39 | 20 | 50 | 69 |
| | | | 20 | 12.5 | 12.5 | 20 | 20 | 26 | 30 | 25 | 42 |
| | | | | | | 30 | 12.5 | 10 | 45 | 18.2 | 20.3 |
| | | | | | | | | | 60 | 12.5 | 10.0 |
| K = 0.13 | | | K = 0.06 | | | K = 0.042 | | | K = 0.021 | | |

TABLE 17

*Synopsis of Table 15*

| Temp. | K (obs.) | K (calc.) |
|---|---|---|
| 56.012° | 0.403 | 0.403 |
| 55.11° | 0.271 | 0.271 |
| 54.012° | 0.167 | 0.170 |
| 53.175° | 0.111 | 0.117 |
| 52.012° | 0.074 | 0.071 |
| 51.06° | 0.05 | 0.046 |
| 50.012° | 0.0295 | 0.0295 |

$\mu - 92600$

TABLE 18

*Synopsis of Table 16*

| Temp. | K (obs.) | K (calc.) |
|---|---|---|
| 58.02° | 0.8 | 0.89 |
| 57.13° | 0.61 | 0.60 |
| 56.02° | 0.455 | 0.36 |
| 55.03° | 0.27 | 0.23 |
| 54.02° | 0.14 | 0.15 |
| 53.13° | 0.13 | 0.10 |
| 52.03° | 0.06 | 0.06 |
| 51.25° | 0.042 | 0.042 |
| 50.13° | 0.021 | 0.025 |

$\mu - 97100$

Tables 17 and 18 give a synopsis of the two preceding experiments and show that temperature coefficient is rather high. Also the two fractions of the complement, the *globulin* fraction and the *albumin* fraction, the so-called "middle piece" and "end piece" follow the same laws.

In the "inactivation" of guinea-pig complement for the Wassermann test an "inactivation temperature" is sometimes spoken of, at which the complement is "killed." This conception is, of course, wrong. As it appears from Table 16 a decrease of the complement's power to 10 takes at 58.02° 1 minute, at 55.03° 4 minutes, at 53.13° 9 minutes, at 51.25° 30 minutes and at 50.13° 60 minutes.

Does the same law apply at lower temperatures as for temperatures above 55°, those we usually employ for the "inactivation" of complement? This can be calculated by means of our formula; we then find that at temperatures below zero an inactivation would take an enormous number of years. We know that this is not the case, and that complement deteriorates both at 2° and even at 14°, as will be seen from Table 19.

TABLE 19

*Destruction of Guinea-Pigs Complement at Different Temperatures*

| 37° | | | 20° | | | 2° | | | —14° | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| t (hours) | q (obs.) | q (calc.) | t (days) | q (obs.) | q (calc.) | t (days) | q (obs.) | q (calc.) | t (days) | q (obs.) | q (calc.) |
| 0 | 200 | 200 | 0 | 200 | 200 | 0 | 200 | 200 | 0 | 200 | 200 |
| 18 | 77 | 77 | 1 | 100 | 92 | 2 | 200 | 171 | 2 | 200 | 174 |
| 24 | 50 | 56 | 2 | 25 | 44 | 4 | 154 | 148 | 4 | 154 | 154 |
| 40 | 25 | 24 | 3 | 15.4 | 18.6 | 6 | 143 | 138 | 6 | 154 | 134 |
| 48 | 12.5 | 16.8 | 4 | 10 | 9 | 8 | 125 | 110 | 8 | 125 | 118 |
| 62 | 10 | 7.6 | | | | 10 | 100 | 94 | 10 | 100 | 104 |
| | | | | | | 12 | 77 | 81 | 12 | 100 | 90 |
| | | | | | | 14 | 63 | 70 | 14 | 77 | 80 |
| | | | | | | 16 | 60 | 60 | 16 | 63 | 70 |
| | | | | | | 18 | 50 | 51.5 | 18 | 63 | 62 |
| | | | | | | 20 | 40 | 44.5 | 20 | 50 | 54 |
| | | | | | | 24 | 33.3 | 33.3 | 24 | 40 | 41.5 |
| | | | | | | 28 | 25 | 24.5 | 28 | 33.3 | 32 |
| | | | | | | 32 | 20 | 18.1 | 32 | 25 | 25 |
| | | | | | | 36 | 10 | 13.5 | 36 | 20 | 19.1 |
| | | | | | | 40 | 7 | 10 | 40 | 12.5 | 14.8 |
| | | | | | | 45 | 5 | 6.9 | 45 | 10 | 10.7 |
| K = 0.00038 | | | K = 0.000234 | | | K = 0.0000226 | | | K = 0.0000196 | | |

The course of the reaction itself follows, likewise, the law of monomolecular reaction (see the calculated values) at these relatively low temperatures, but the influence of temperature on the rate of reaction is not nearly as important as at higher temperatures. In Table 20 is indicated, first, the rate of destruction of

TABLE 20

*Guinea-Pigs Complement. Rate of Destruction at Different Temperatures*

| Temp. | K (obs.) | K (calc.) |
|---|---|---|
| 55.05° | 0.31 | 0.38 |
| 54.01° | 0.22 | 0.22 |
| 52.99° | 0.14 | 0.13 |
| 52.19° | 0.09 | 0.087 |
| 51.11° | 0.05 | 0.05 |
| 50.09° | 0.029 | 0.029 |
| 37° | $3.5\times10^{-4}$ | $2.4\times10^{-5}$ |
| 20° | $1.1\times10^{-4}$ | $8.9\times10^{-10}$ |
| 2° | $3.2\times10^{-5}$ | $4.6\times10^{-15}$ |
| −14° | $1.6\times10^{-5}$ | $2.2\times10^{-20}$ |

$\mu = 109000$

guinea-pigs complement at temperatures from 55.05° to 50.09° (K obs.). These values follow the law of van't Hoff-Arrhenius, as shown by the calculated values (K calc.). The temperature coefficient was found to be 109000. By means of this law we could calculate what the values at lower temperatures, *e.g.*, 37°, 20°, 2°, and −14°, should theoretically be. The values are indicated above in the frame. The comparison with the figures really observed shows a marked difference; these figures are, in fact, very much smaller, *e.g.*, at 2° the rate of destruction observed being $3.2 \times 10^{-15}$, whilst, according to calculation, it should only be $4.6 \times 10^{-5}$. The observed rate of destruction being thus $10^{-10}$ higher. At −14 the corresponding figures are $1.6 \times 10^{-5}$, instead of, as calculated, $2.2 \times 10^{-20}$.

It appears from these experiments that the temperature coefficient is much higher at high than at low temperatures; the

same thing was true, as above stated, in the case of the diphtheria antitoxin.

Also at temperatures between 50° and 60° the temperature coefficients in the different experiments are rather variable. The examination of 24 different complements showed values for $\mu$ between 44000 to 114000, about 86000 on an average. This means that each time the temperature is raised 1 degree, the rate of destruction will be increased one and one-half times.

The other part of the hæmolytic system, the *amboceptors,* are much more thermostabile than the complement. This applies both to the amboceptor, which we find in normal serum and to those obtained by immunization. The destruction of all the amboceptors we hitherto have examined can be expressed by the monomolecular law and the rate of destruction at different temperatures follows the law of van't Hoff-Arrhenius.

I shall first mention an example of *normal* amboceptor from swine; the serum was diluted with physiological sodium chlorid to prevent coagulation.

TABLE 21

*Destruction by Heat of Amboceptor from Normal Swine Serum.*
*(Serum Diluted 1:3)*

| 72.04° | | 70.05° | | | 68.12° | | | 66.04° | | |
|---|---|---|---|---|---|---|---|---|---|---|
| t (min.) | q (obs.) | t (min.) | q (obs.) | q (calc.) | t (min.) | q (obs.) | q (calc.) | t (min.) | q (obs.) | q (calc.) |
| 0 | 25 | 0 | 25 | 25 | 0 | 40 | 40 | 0 | 50 | 50 |
| 1 | 3.3 | 1 | 10 | 10 | 3 | 12.5 | 14 | 5 | 20 | 25 |
| | | 2 | 4 | 4 | 6 | 5 | 5 | 10 | 12.5 | 12.5 |
| | | | | | 9 | 2.5 | 1.8 | 15 | 6.25 | 6.25 |
| | | | | | | | | 20 | 3.3 | 3.2 |
| K=0.876 | | K=0.298 | | | K=0.15 | | | K=0.06 | | |

Table 22 presents the destruction of an *immune-amboceptor* from a *rabbit,* immunized with an emulsion of sheep erythrocytes, the amboceptor generally used for the Wassermann

TABLE 22

*Destruction at Different Temperatures of Immunamboceptors from Serum of Rabbit, Immunised with Sheep's Blood Corpuscles. (Serum Diluted 1:19)*

| 80.18° | | | 78.10° | | | 76.05° | | | 74.12° | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| t (min.) | q (obs.) | q (calc.) | t (min.) | q (obs.) | q (calc.) | t (min.) | q (obs.) | q (calc.) | t (min.) | q (obs.) | q (calc.) |
| 0 | 200 | 188 | 0 | 250 | 250 | 0 | 250 | 268 | 0 | 400 | 400 |
| 1 | 125 | 125 | 1 | 154 | 160 | 1 | 200 | 200 | 1 | 333 | 320 |
| 2 | 76.9 | 82 | 2 | 125 | 116 | 2 | 154 | 150 | 2 | 250 | 250 |
| 3 | 50 | 54 | 3 | 77 | 79 | 3 | 125 | 113 | 3 | 200 | 200 |
| 4 | 33.3 | 36 | 4 | 50 | 54 | 4 | 100 | 85 | 4 | 154 | 158 |
| 5 | 25 | 24 | 5 | 33.3 | 37 | 5 | 62.5 | 64 | 5 | 125 | 125 |
| 6 | 20 | 16 | 6 | 25 | 25 | 6 | 50 | 48 | 6 | 100 | 100 |
| | | | 7 | 20 | 17 | 7 | 33.3 | 36 | 7 | 62.5 | 79 |
| | | | | | | 8 | 25 | 26.5 | 8 | 40 | 63 |
| K = 0.178 | | | K = 0.167 | | | K = 0.128 | | | K = 0.1 | | |

| 72.10° | | | 70.15° | | | 68.08° | | | 66.12° | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| t (min.) | q (obs.) | q (calc.) | t (min.) | q (obs.) | q (calc.) | t (min.) | q (obs.) | q (calc.) | t (min.) | q (obs.) | q (calc.) |
| 0 | 625 | 625 | 0 | 1000 | 800 | 0 | 2000 | 2000 | 0 | 2500 | 2500 |
| 2 | 400 | 400 | 2 | 500 | 500 | 3 | 1180 | 1200 | 5 | 1540 | 1400 |
| 4 | 200 | 250 | 4 | 250 | 320 | 6 | 1000 | 720 | 10 | 769 | 790 |
| 6 | 125 | 155 | 6 | 154 | 200 | 9 | 400 | 430 | 15 | 500 | 445 |
| 8 | 100 | 98 | 8 | 125 | 129 | 12 | 250 | 260 | 20 | 250 | 250 |
| 10 | 62.5 | 61 | 10 | 100 | 82 | 15 | 154 | 154 | 25 | 125 | 139 |
| 12 | 40 | 38 | 12 | 50 | 52 | | | | 30 | 76 | 79 |
| 14 | 25 | 23.9 | 14 | 33 | 33 | | | | 40 | 33 | 25 |
| | | | 16 | 25 | 21 | | | | | | |
| K = 0.101 | | | K = 0.099 | | | K = 0.0737 | | | K = 0.05 | | |

reaction. Furthermore, the hæmolytic serum, indicated by Forssman, obtained by treating rabbits with guinea-pig kidney is examined.

TABLE 23

*Destruction by Heat of Immunamboceptor, Prepared by Treating Rabbits with Guinea-Pig Kidney (Serum Diluted 1:9).*

| 80.12° | | | 78.08° | | | 76.07° | | | 74.05° | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| t (min.) | q (obs.) | q (calc.) | t (min.) | q (obs.) | q (calc.) | t (min.) | q (obs.) | q (calc.) | t (min.) | q (obs.) | q (calc.) |
| 0 | 100 | 100 | 0 | 100 | 100 | 0 | 125 | 120 | 0 | 143 | 143 |
| 1 | 77 | 68 | 1 | 77 | 71 | 1 | 111 | 96 | 1 | 125 | 118 |
| 2 | 44.4 | 47 | 2 | 50 | 51 | 2 | 77 | 75 | 2 | 111 | 97 |
| 3 | 28.6 | 32.5 | 3 | 33.3 | 37 | 3 | 50 | 59 | 3 | 100 | 80 |
| 4 | 23.5 | 22.5 | 4 | 25 | 26.5 | 4 | 40 | 47 | 4 | 57.1 | 65 |
| | | | 5 | 21 | 19 | 5 | 33.3 | 37 | 5 | 44.4 | 53 |
| | | | | | | 6 | 27 | 29.5 | 6 | 36.4 | 44 |
| | | | | | | 7 | 23.5 | 23 | 7 | 33.3 | 36 |
| | | | | | | 8 | 20 | 18 | 8 | 28.6 | 29 |
| | | | | | | | | | 10 | 25 | 20 |
| K = 0.162 | | | K = 0.145 | | | K = 0.103 | | | K = 0.085 | | |

| 72.10° | | | 70.05° | | | 68.10° | | | 66.02° | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| t (min.) | q (obs.) | q (calc.) | t (min.) | q (obs.) | q (calc.) | t (min.) | q (obs.) | q (calc.) | t (min.) | q (obs.) | q (calc.) |
| 0 | 154 | 154 | 0 | 200 | 200 | 0 | 286 | 286 | 0 | ? | 220 |
| 2 | 125 | 119 | 2 | 143 | 143 | 3 | 167 | 185 | 5 | 154 | 154 |
| 4 | 87 | 92 | 4 | 111 | 112 | 6 | 125 | 120 | 10 | 111 | 109 |
| 6 | 77 | 73 | 6 | 100 | 90 | 9 | 77 | 78 | 15 | 63 | 76 |
| 8 | 56 | 56 | 8 | 67 | 72 | 12 | 50 | 51 | 20 | 44 | 53 |
| 10 | 44 | 43.5 | 10 | 50 | 53.5 | 15 | 33.3 | 33.3 | 25 | 33.3 | 37.5 |
| 12 | 33.3 | 34 | 12 | 40 | 45 | 20 | 22.2 | 16.3 | 30 | 26.7 | 26.7 |
| 14 | 26.7 | 26.7 | 14 | 33.3 | 36 | | | | 40 | ? | 13 |
| | | | 16 | 28.6 | 28.6 | | | | | | |
| | | | 20 | 23.5 | 20 | | | | | | |
| K = 0.054 | | | K = 0.048 | | | K = 0.062 | | | K = 0.0307 | | |

It appears that in all cases the temperatures suitable for measuring the rate of destruction were between 66° and 80°, thus being much higher than the inactivation temperature of the complement.

If we examine the influence of changes of temperature on the rate of destruction we find great variations. The synopsis, given in Table 24,

TABLE 24
*Synopsis of*

| *Table 21* | | | *Table 22* | | | *Table 23* | | |
|---|---|---|---|---|---|---|---|---|
| Temp. | K (obs.) | K (calc.) | Temp. | K (obs.) | K (calc.) | Temp. | K (obs.) | K (calc.) |
| 72.04° | 0.876 | 0.876 | 80.18° | 0.178 | 0.178 | 80.12° | 0.162 | 0.17 |
| 70.05° | 0.298 | 0.36 | 78.10° | 0.167 | 0.154 | 78.08° | 0.145 | 0.132 |
| 68.12° | 0.15 | 0.15 | 76.05° | 0.128 | 0.133 | 76.07° | 0.103 | 0.104 |
| 66.04° | 0.06 | 0.058 | 74.12° | 0.1 | 0.116 | 74.05° | 0.085 | 0.080 |
| | | | 72.10° | 0.101 | 0.10 | 72.10° | 0.054 | 0.064 |
| | | | 70.15° | 0.099 | 0.086 | 70.05° | 0.048 | 0.05 |
| | | | 68.08° | 0.074 | 0.074 | 68.10° | 0.062 | 0.039 |
| | | | 66.12° | 0.05 | 0.063 | 66.02° | 0.0307 | 0.0307 |
| $\mu = 105900$ | | | $\mu = 17560$ | | | $\mu = 29050$ | | |

of the three preceding experiments, shows that they follow quite well within the errors of experiment the van't Hoff-Arrhenius law; but the temperature coefficient varies considerably, from 17560 to 105900. A number of other experiments have hitherto given us no governing law, as the following table shows:

TABLE 25

| | | $\mu$ |
|---|---|---|
| Normal swine serum | 1 | 106400 |
| Normal swine serum | 2 | 105900 |
| Normal swine serum | 3 | 60160 |
| Immun-serum rabbit | 1 | 104700 |
| (Sheep corpuscles) | 2 | 68340 |
| (Sheep corpuscles) | 3 | 24060 |
| (Sheep corpuscles) | 4 | 17560 |
| Immun-serum rabbit | 1 | 29050 |
| (Guinea-pig kidney) | 2 | 16190 |

It is not only to dissolved substances that the laws of thermo-chemistry can be applied, but also to phenomena, such as hæmolysis, agglutination, disinfection and phagocytosis; time does not permit us to enter into details, but it must be emphasized that *cells* taken from living organisms have many peculiar qualities in relation to variations of temperature. Thus the *white blood-corpuscles* bear the impress of the organism from which they are taken. The maximum of their phagocytic power occurs exactly at the same temperature as that of the mother organism, as appears from Table 26:

TABLE 26

| | Body temperature | Maximum of phagocytic power |
|---|---|---|
| Man | 37° | 37° |
| Horse | 37.8° | 38° |
| Guinea-pig | 39° | 39° |
| Hen | 41° | 41° |
| Pigeon | 41° | 41° |

This maximum varies even in the same individual, *e.g.*, during fever; variations in the body temperature will produce exactly corresponding variations in the phagocytic optimum.

With the preceding remarks I have tried to give a short survey of the significance of temperature on immuno-chemical reactions, illustrated by a few examples. Further details may be found in the publications of the State Serum Institute. I hope that I have been able to show that many of these processes follow simple laws; the destruction by heat can in most cases be expressed by mono- or bimolecular law, and its relation to varying temperatures by the exponential law of van't Hoff-Arrhenius. It is remarkable that the temperature coefficient generally is very elevated, so that within a certain zone of temperature, different for different bodies, a very small change of temperature, one degree or less, may produce an enormous difference in the rate of destruction. This zone, popularly called the "inactivation" temperature, is on the average lower for the antigens, such as toxins and complements, higher for the antibodies, such as antitoxins, agglutinins, amboceptors. But the temperature coefficient is exceedingly variable, not only for bodies, which are supposed to be identical, as is shown above for the amboceptors (Table 26), but even for the serum substance. Thus Walbum and I found for rennet that the same preparation which in September had a temperature coefficient of 91200, two months later showed a coefficient of 18500.

But even if we are only in the beginning of our investigation in this field, it is encouraging that the reactions we hitherto have studied seem to follow the same laws as are valid for other chemical processes.

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(See Communications de l'Institut sérothérapique de l'Etat Danois.)

# THE RELATION OF MECHANICAL AND ELECTRICAL PHENOMENA OF MUSCULAR CONTRACTION, WITH SPECIAL REFERENCE TO THE CARDIAC MUSCLE [1]

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THE subject which I have chosen for this lecture concerns itself with the relationship between the mechanical and electrical phenomena which occur when a muscle contracts. Every

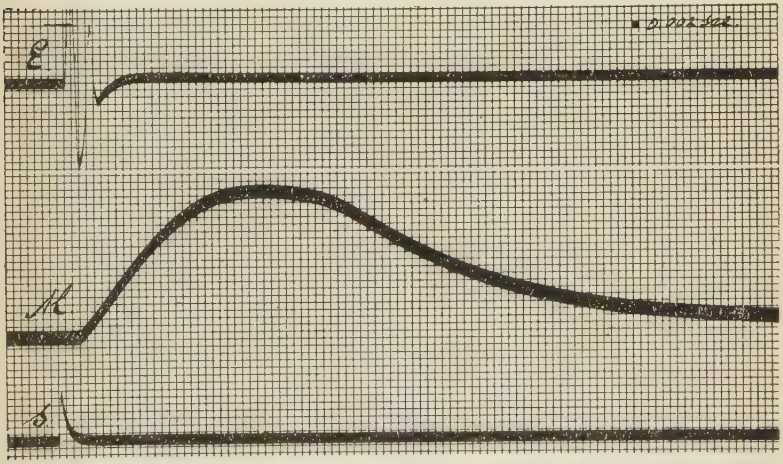

FIG. 1.—Gastrocnemius muscle of a frog stimulated by a single induction shock through the sciatic nerve. *E*, electromyogram; *M*, mechanomyogram; *S*, signal. *Hoogerwerf.*

muscle when contracting develops an electrical current which, indeed, is very weak, if you compare it with the current of an ordinary incandescent lamp, but which, nevertheless, may be measured and recorded very well by means of sensitive instruments.

The curves obtained are called *electromyograms* and are valuable means of studying the true nature of a muscular contraction.

[1] Lecture delivered December 6, 1924.

We shall deal with muscles in general and the cardiac muscle in particular.

When a muscle is stimulated by a single induction shock and is made to register both the mechano- and electromyogram, the curves obtained have the appearance shown in figure 1. The upper curve shows the electrogram recorded by the galvanometer; next below is the mechanogram, then follows the signal indicating the moment of stimulation.

The mechanogram is obtained by using a lever which is connected to the muscle by means of a spring. Every increase in tension of the muscle produces a movement of the lever. The muscle shortens a little, too, but this shortening is so trifling that we practically may say that the muscle increases its tension exclusively. The mode of contraction is called isometric.

A distinctly measurable time is seen to pass between the moment at which the electrogram makes its appearance and that at which the mechanogram begins to develop.

The generally accepted interpretation of the curves shown to you is as follows: the stimulus causes the development of an excitation wave which, starting from the stimulation-point, is propagated through the muscle and is the real cause of the electric current. A short time after the excitation-wave and after the development of the electric current, the mechanical action in the muscle starts; it either increases its tension or it shortens.

If you consider the curve as representing the true time relations, the interpretation given is quite conceivable,—it may, in fact, be the only possible one. There is a well-known comparison between the muscle and a steam engine. The mechanical power developed by the engine is consequent to the passage of the steam through the steam pipe. First the valve in the steam pipe has to be opened. The electric current in the muscle should be comparable to the movement of the valve, the mechanical power developed in the muscle comparable to the mechanical power of the engine.

The cardiac muscle differs in many respects from an ordinary skeletal muscle, but the principles which rule its contraction are

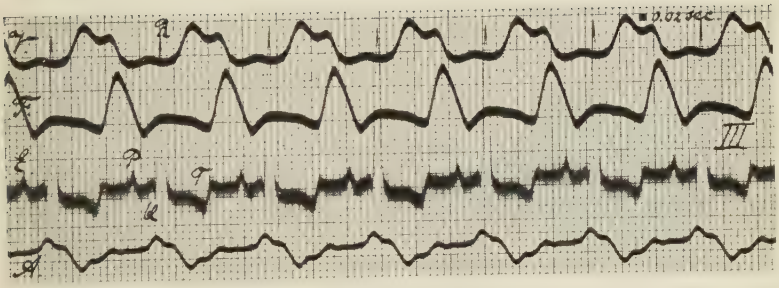


FIG. 2.—Dog. *V*, variations of pressure in the left ventricle; *A*, variations of pressure in the right auricle; *F*, pulse of the femoral artery; *E*, electrocardiogram, lead III. *Dasbach.*

the same. With every systole there is, besides the mechanical effect, the development of an electric current, and on ordinary records we can see that the electrical phenomenon precedes the development of the mechanical power.

The upper curve of figure 2 represents the variations of pressure in the left ventricle. A tube is introduced by way of the carotid artery into the left ventricle of a dog. The variations in pressure are recorded by a lever.

In a similar way the variations in pressure of the right auricle *A*, and of the arterial pulse *F* are recorded, whereas the third curve *E* represents the galvanometric record of the electric currents, developed by the cardiac muscle. The P-wave corresponds to the electric current of the auricles. You see that it precedes their increase in pressure to an amount of 2 or 3 millimetres, each millimetre corresponding to the time of 0.02 sec.

The peak *Q* and the large peak *R* correspond to the contraction of the ventricles. They precede the systole also about two millimetres.

Of course, the pulse-wave is very much later, because of the time required to propagate the wave from the heart to the artery, the pulse of which is recorded.

The comparison with the steam engine and its valve seems to be as well applicable to the heart as to the ordinary skeletal muscle. According to this view, there would only be an indirect connection between the contraction and the electric current. If there is no steam in the boiler, you may open the valve of the steam pipe, and there will be no movement of the engine. In very much the same way it might be possible, for instance in pathological cases, or by means of a poison or drug or in some other way, to inhibit or even to block the transition between the excitation wave and the contraction, and thus completely separate the two phenomena.

I shall now try to demonstrate that this view is based on insufficient evidence and that a different explanation is possible. According to the latter, the process which causes the increase of tension in the muscle is itself the cause of the electric current,

and thus the electrical and mechanical phenomena are closely and inseparably connected.

At first sight this supposition seems to have very little to recommend it. The curves shown to you make it difficult to believe that two processes differing so much in time and in form should in reality be so closely coupled.

Now let us first inquire, whether in forming our opinion we may assume that the recorded curves are reliable representations of the true facts, and whether very much weight may be attached to the apparent time-interval between the electrical and mechanical phenomena. We must take into account that the registration of a movement is bound to involve some loss of time. When a body at rest of mass $m$ is set in motion by a force $f$, the distance described by the body will be given by the formula

$$s = \frac{1}{2}\frac{f}{m}t^2$$

$t$ representing the time.

The distance $s$ represents the displacement of the marking lever and that is the quantity which can be made visible and registered. We see from the formula that when the force is small and the mass large, or rather when the ratio of the force to the mass is small, the distance $s$ is also small, and we know moreover that some time is required for the force in a muscle to rise to a certain value. This development of force, which follows the stimulus, is a vital process and, like all vital processes, proceeds slowly and gradually. If we assume that during the first few thousandths of a second after the stimulus the force increases proportionately to $t$, we must conclude that the distance $s$, described by the lever, is proportional to $t^3$. And thus we see that for very small times the displacement of the lever may be imperceptible, at least by means of the instruments at our disposal.

The electrogram is less retarded than the mechanogram, since in that case the mass of the body which has to be set in motion, say the string of a string-galvanometer or the small column of mercury of the capillary electrometer, is very small in relation to the acting force.

If we don't take the utmost care in obtaining the mechanical response of a muscle as soon as possible after the stimulation, the shortening observed may be retarded enormously. This is, for instance, the case when we record an isotonic contraction.

If we try to obtain the visible contraction of the muscle as soon as possible, we meet with many technical difficulties. As the technique has improved in the course of years, the results of

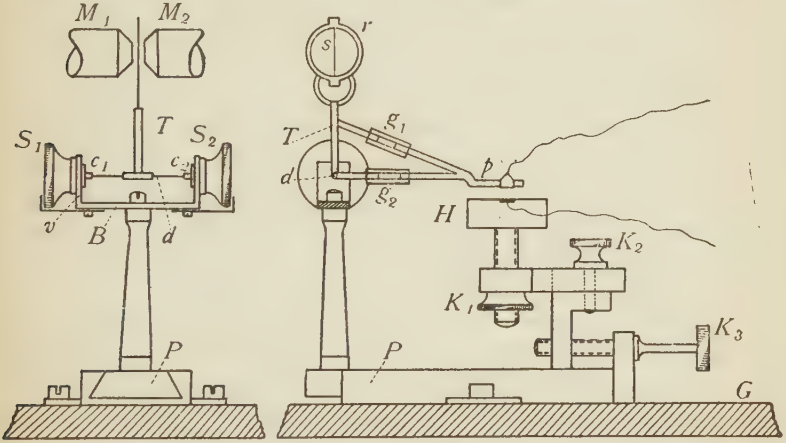


FIG. 3.—String myograph. *d*, stretched metal wire to which a right-angled triangle *T* $g_1$ $g_2$ is attached. The muscle rests on the stage *H*. A quartz fibre *s* is mounted in a ring *r* and placed between the microscopes $M_1$ and $M_2$.

the measuring experiments have had to be revised. Subsequent investigators have found shorter and shorter latent periods.

*Helmholtz* found that the latent period of muscular contraction, *i.e.*, the time elapsing between the excitation and the visible contractile process, amounted to about one-hundredth of a second. Later it was shown by Burdon Sanderson that under favorable conditions this interval need not be more than 0.003 of a second ($3\sigma$). Koranyi and Vas tested isolated muscular fibres microscopically, and using a stroboscopic method, showed that the latent period could not exceed 1 $\sigma$.

For measuring the latent period we have used the following device (see diagram, Fig. 3). *d* is a stretched metal wire to which a right-angled triangle is attached. The muscle rests on a

stage $H$. On contraction it becomes thicker and thus makes the triangle turn. At the top of its vertical side a ring $r$ is attached in which a fine quartz-fibre $s$ which has been made opaque is mounted. This fibre is placed between two microscopes, $M_1$ and $M_2$. The action of the triangle is by torsion and completely free of friction.

A magnification of 1000 to 2000 diameters was used, and in order to make $f$ as large as possible we always worked with a fresh muscle, which was brought to its maximum contraction by a strong induction-shock. In this manner we found for the latent period about the same value as Koranyi and Vas, *viz.*, 1 $\sigma$.

Before drawing conclusions from this result, there are some points to be considered. First of all, we must remember that our apparatus is not ideal. If the mass of the triangle, in itself very small, could be reduced still further, and particularly if we could use still higher magnifications, we should undoubtedly find even smaller values for the latent period.

A second point to which we have to draw attention is the duration of the stimulus. An electric current, as used for the stimulation, can only produce an effect after having passed for some time and thus having accumulated a certain amount of energy. The stronger the current, the shorter the time required. By means of a rapidly registering measuring instrument we have been able to establish that the induction current used by us, which was derived from a simple small coil, lasts more than two-thousandths of a second. A not altogether negligible portion of this time is required for the stimulus to become effective, and thus we must conclude that the actual latent period is shorter than what is given by our registration. The interval between the electrical and the mechanical reactions in the muscle is bound to be even less, for on general physiological grounds we cannot but suppose that the electrical reaction, as also being a vital process of the muscle, requires some time to establish itself.

Taking all this into account, it does not seem at all impossible, or perhaps we may even say it becomes probable, that the two reactions develop simultaneously. In any case the apparent interval found, when the measurements are made with less per-

fect instruments, cannot be used as an argument in favor of the view that the two reactions which follow one and the same stimulus are two separate and separable processes.

The above experiments were made with the gastrocnemius muscle of the frog. If it is replaced by a nonstriated muscle, like that of the stomach of a frog, the latent period is found to be longer. This must be explained by the circumstance, that the processes in a nonstriated muscle are much slower, and that therefore the development of force, after the stimulus has been applied, takes place relatively slowly; $f$ in our formula is small, hence $s$ the displacement in the first few thousandths of a second is also small. That the latent period of a frog's stomach should reach 14 seconds, as given by some investigators is an estimate wide of the mark. In our measurements we found intervals of less than a tenth of a second, and a duration of that magnitude we think can be reconciled with the view entertained by us, since it can be entirely explained by the imperfections of our instruments.

We shall now discuss measurements made on the spontaneously beating heart, where the electrical and mechanical phenomena may be registered simultaneously, without fear of a possible current escape from the stimulus itself. By means of a device, similar to that used in the investigation of the gastrocnemius muscle of the frog, we have made measurements on the heart of the frog and of the rat. If the lead from the heart is not the most appropriate, it may happen that the electric wave at the beginning of its development is situated exactly half way between the two electrodes, in consequence of which no current is registered by the galvanometer. In that case the instrument does not indicate a current until later, when the wave has traveled a certain distance.

It is not difficult to realize the above-mentioned conditions and to register curves from a frog's heart, which show the mechanogram a considerable time before the electrogram.

For the purpose of avoiding the Eg being late, we injured in the rat's heart the apex of the ventricle over some distance and led off from the point of lesion and from a place near the base.

The demarcation-current arising was compensated in the usual way.

If we might assume that the stimulus, on its way to the ventricle along the bundle of His first reaches some of the fibres whose ends have been injured, then the negative wave, which is formed, would undoubtedly become visible in the galvanometer-curve in its first phase. The demarcation-current would immediately show its negative fluctuation. But the muscular fibres of which the heart consists have different directions and but a limited length, so that we cannot exclude the possibility of the fibres which have remained intact being first affected by the stimulus. It might be that the negative wave was found for a short space at a spot symmetrically situated with respect to the electrodes, and thus the Eg might STILL come late. That this actually happens is shown by the curves of figure 4. For if the mechanogram is registered under favorable circumstances, we may see it appear before the Eg.

By "favorable" circumstances we mean a strong magnification, say of 1000 diameters, and the force developed, the *f* of the formula as high as possible. The latter is accomplished by using a fresh, strongly beating heart.

Time-measurements can also be made with the human heart, the best method being to replace the mechanocardiogram by a registration of the heart's sounds, the phonocardiogram.

In figure 5 you see the Ecg and the apex sounds of a man recorded simultaneously. In the first sound we distinguish three parts, *viz.*, *a*, the initial vibrations being a muscular sound caused by the contraction of the muscular wall of the heart, *b* the main vibrations, *c* the end vibrations. In figure 6 you see another instance taken under the same conditions. The initial vibrations appear practically at the same time or only some few thousandths of a second after the beginning of the Ecg. The most probable conclusion is that the heart sounds actually set in at exactly the same moment as the Ecg, because the time which is needed to transfer the sound from the heart through the

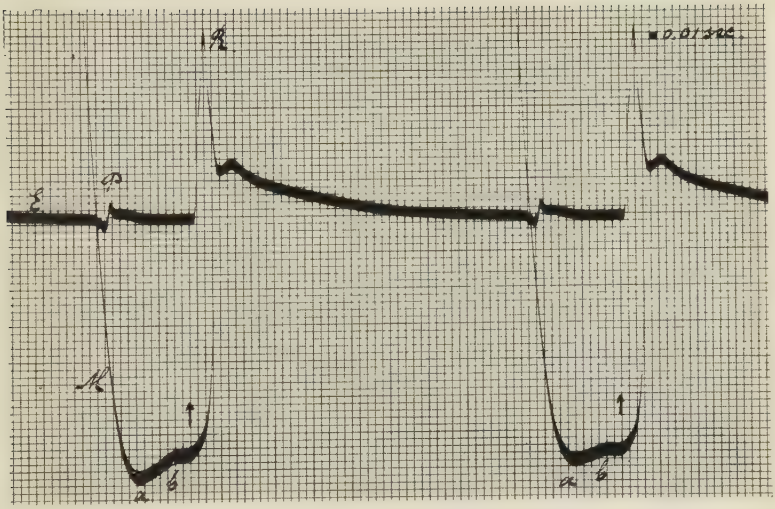

FIG. 4.—Isolated heart of a rat. *E*, electrogram; *M*, mechanogram. The arrow indicates the moment when the upstroke of the mechanogram begins. *de Jongh*.

FIG. 5.—*Ph*, apex sounds and *E*, electrocardiogram of a man; 1, 2, 3, first, second, and third sound; *a*, *b*, *c*, the three parts of the first sound. The arrow indicates the beginning of the sounds as well as of the ventricular complex of the Ecg. *Battaerd*.

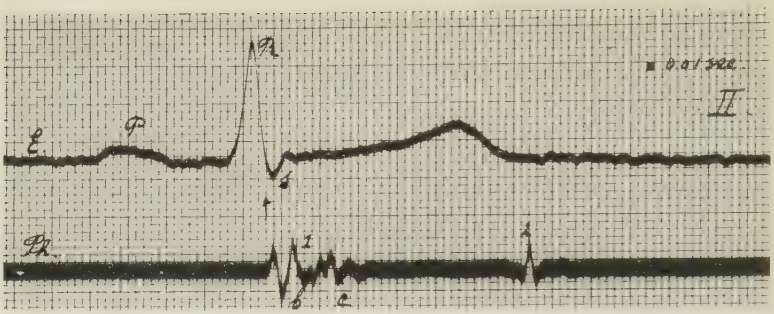

Fig. 6.—Another instance taken under the same conditions as Fig. 5. *Battaerd.*

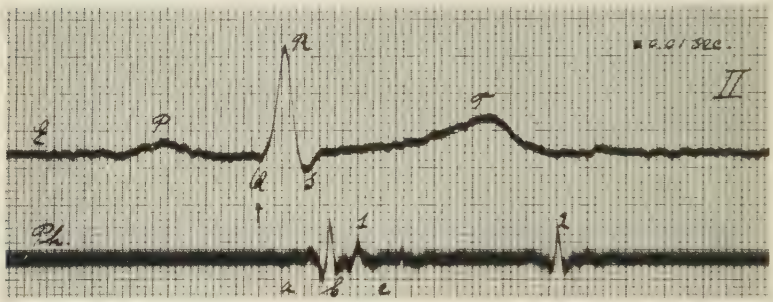

Fig. 7.—Another cycle of the same person as that of Fig. 6, taken on the same photographic plate. Here the initial vibrations of the apex sound are very weak, so that they are practically imperceptible. Therefore the sound is late with respect to the Ecg. *Battaerd.*

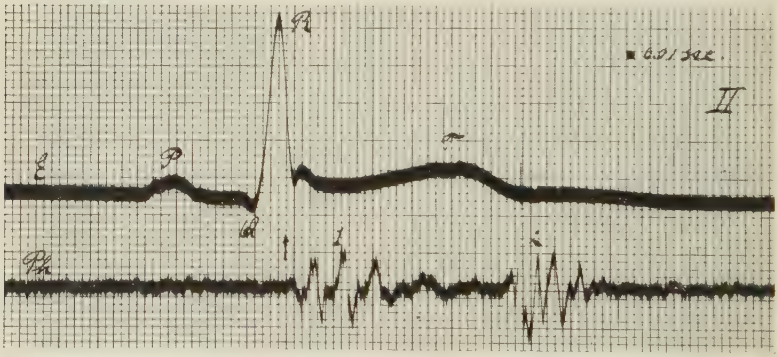

Fig. 8. –The heart sounds are taken over the valve of the pulmonary artery. They are late. *Battaerd.*

stethoscope to the microphone must be taken into account. With our apparatus, in which the sound traveled a distance of about 75 cms, the time in question amounted to 2.2 $\sigma$.

If the recording apparatus is not sufficiently sensitive, so that the initial vibrations are not visible, or if the stethoscope is not placed over the apex, the vibrations are late. This is shown in the figures 7 and 8.

Some physiologists hold the view that we, a priori have to assume, that the electric phenomena precede the mechanic ones. In nerve we have an electric response without a mechanic one. And nerve and muscle belong together. Therefore, if we don't find a difference of a 0.001 sec, there may be a difference of a 0.0001 sec. But, at any rate, there must exist a difference.

I cannot agree with this standpoint.

Physiology is an inductive science. Every physiological theory has to be based on facts, and if the facts are not well proved, we have no sufficient evidence for the theory.

There are still three groups of phenomena to be discussed: in the first place the incongruity between the electro- and mechanogram, a lack of parallelism between the two curves; in the second place, *Gaskell's* experiment, and, finally, the attempts which have been made to separate the mechanical and the electrical phenomena in the systole by means of various drugs.

First, the incongruity referred to. It is, indeed, striking, but its explanation is not difficult. We must remember that the conditions under which a contraction can be made visible, for instance, by the movement of a lever or a marker, are totally different from those for the registration of a current. The latter requires the presence of a potential-difference between two parts of the muscle, which, in its turn, must be caused by a difference in composition, in action or in metabolism. The one part may, for instance, be in condition of contraction, the other in that of relaxation. But if the whole muscle is in the same condition, either of contraction or of relaxation, and all local differences are removed, there can be no potential-difference, and the gal-

vanometer will be free of current. We shall illustrate this with a few instances.

The above diagram represents a muscle which is stimulated at one end. The stimulus causes a contraction-wave to run along the muscle, beginning at the place of stimulation and proceeding to the other extremity of the muscle. The current is led to the galvanometer from *a* and *b*. At first, *a* becomes negative with respect to *b*, and afterwards with respect to *a*, and the galvanometer will register a diphasic current (see Fig. 10).

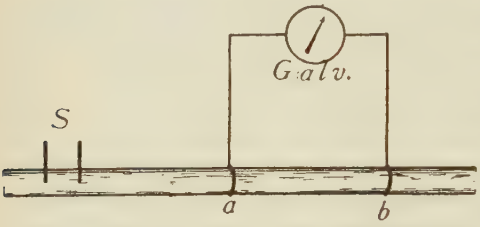


Fig. 9.—Diagram showing a muscle stimulated at *S*. The action current is led to the galvanometer from *a* and *b*.

At the moment when the current changes from the one to the other direction, that is to say, about the middle of the curve, the muscle is in full contraction, although no current can be registered at this moment. Here we have an instance showing how in a definite phase of the muscular action a contraction may exist, without being accompanied by an electric current. In this case the dissimilarity is easily understood and nobody would infer from this that there is no connection between the electric and the mechanical phenomenon.

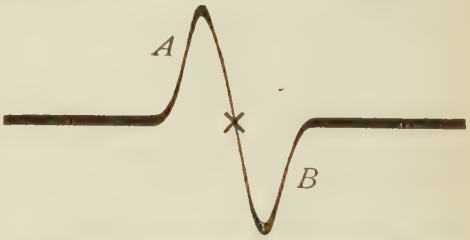


Fig. 10.—Diagram of a diphasic action current. Between the positive phase *A* and the negative one *B* there must be a moment of equipotentiality, although the muscle is in full contraction.

In the case of tetanus, we have the contraction waves blended, the curves of the Ecg and the heart sounds taken simultaneously.

With regard to the duration of the processes there is in general a parallelism that is rather satisfactory. This is shown by the curves of the Ecg and the heart sounds taken simultaneously. The duration of the systole corresponds to the time which elapses

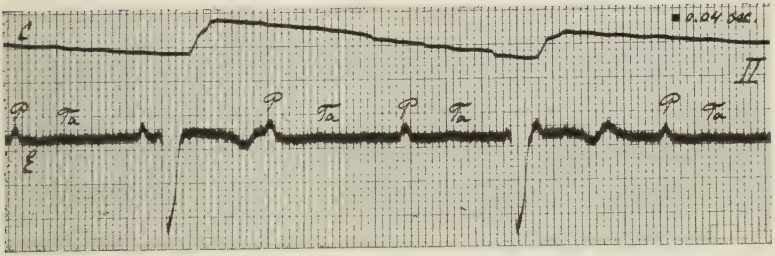


FIG. 11.—*Stokes Adams* disease with preponderance of the left ventricle. *C*, pulse of the carotid artery; *E*, electrocardiogram. *P* $T_a$, auricular complex. The $T_a$-wave is positive.

from the beginning of the first sound to the beginning of the second one, and this corresponds exactly to the duration of the ventricular complex of the Ecg, that is to say, from the beginning of the QRS-group to the end of the T-wave.

Sometimes there seems to be no parallelism. Let us consider the auricle which in the ordinary electrocardiograms seems to produce a much too short electric response. But if we have the opportunity to study the true form of the auricular complex more closely, for instance, in cases of heart block, we find the duration of the action currents considerably longer. There often

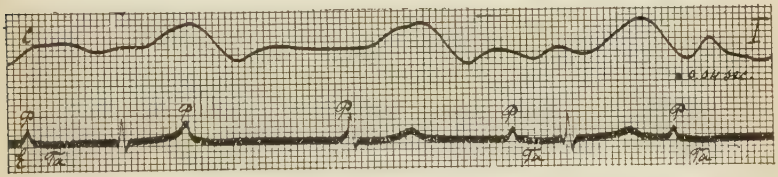

FIG. 12.—*Stokes Adams* disease. *P* $T_a$, auricular complex. The $T_a$-wave is negative.

develops after the P-summit an inconspicuous low second wave, which Hering has called $T_a$-wave. In figure 11 the $T_a$-wave is positive, in figure 12 it is negative.

Another instance of an apparent lack of parallelism between the duration of the mechanical and the electrical processes is to be found in the ventricular complex itself. In figure 13 we reproduce two electrocardiograms of the same person. The upper curve is taken from lead I. The duration of the ventricular systole is measured from the beginning of the QRS-group to the end of the T-wave and in this person amounted to 0.4 second. The bottom curve is taken from the same person but from lead III. In many persons by this lead the T-wave is positive, *i.e.*, directed upwards, in others it is negative, in yet others $T_I$ and $T_{II}$ are about equal, whence $T_{III}$ practically disappears, as is the case with the present subject.

We therefore perceive here an Eg of short duration, about 2.5 scale divisions, or 0.1 second, whereas we know that the systole lasts 10 scale divisions or 0.4 of a second. The appearance of the bottom curve of the figure agrees completely in principle with

the Eg of the gastrocnemius muscle of a frog: a short fluctuation of current, and during the rest of the contraction a nearly straight line.

The similarity is even closer than might be supposed on a superficial inspection of the curves, since also the Eg of the gastrocnemius muscle after the first oscillation shows a line which is not perfectly straight. In many cases an inconspicuous, slow, long-continued negative deflection could be perceived, which may be compared with the T-summit of the Ecg and which stops at about the moment that the contraction ceases (see Fig. 14).

We must return to our Ecg.

During the QRS-group there are phases in which the curve passes through the zero-line, which means that at these moments with a strongly developing systole there is no electric current. The same happens between the QRS-group and the T-summit. The line joining the two, often for a considerable length, coincides with the zero-line. This is especially the case with hearts, whose systole has been lengthened by the action of some drug.

The figure 15 here shown contains at its upper curve the mechanogram, in the second curve the electrogram of a frog's heart poisoned by veratrine. We see the duration of the systole considerably lengthened to 8 or 10 seconds. The Eg has the same length and, as in the human Ecg, we may distinguish at the beginning of the systole the QRS-group, at the end the T-wave. With continued poisoning the duration of the systole increases to about half a minute.

Between the QRS-group and the T-wave the line of the Eg practically coincides with the zero-axis and thus displays a striking want of similarity with the mechanogram. But when we consider what the conditions are, to which the development of an electric and a mechanical force are subjected, we can explain the difference readily. In the curious form of the Eg—the QRS-group at the beginning and the T-summit 30 seconds later at the end of the systole—we may even see a striking confirmation of the view, that a close connection exists between contraction and action-current.

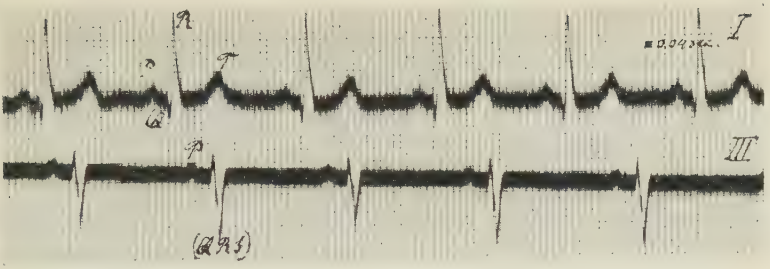

Fig. 13.—Two electrocardiograms of the same person. The upper curve is taken from lead I, the bottom curve from lead III. In the latter curve the *T*-wave practically disappears, so that the duration of the ventricular complex is reduced to 0,1 sec.

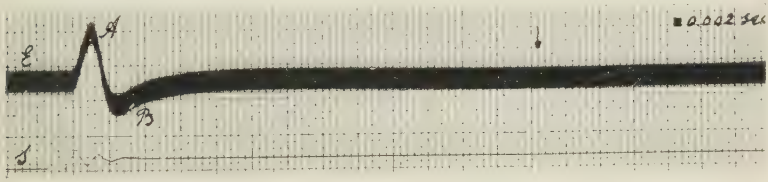

Fig. 14.—Diphasic action current of the gastrocnemius muscle of a frog. *E*, electromyogram; *S*, signal. After the two phases *A* and *B*, there is to be seen an inconspicuous, slow, long continued negative deflection, the top of which is indicated by an arrow. *Battaerd.*

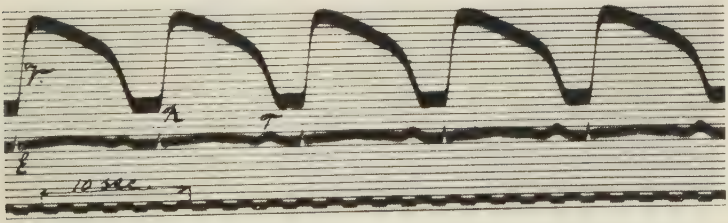

Fig. 15.—Frog's heart poisoned with veratrin. *V*, ventricular mechanogram; *E*, ventricular complex of the electrogram. The duration of the mechano- and of the electrogram is equally lengthened. *v. Lawick v. Pabst.*

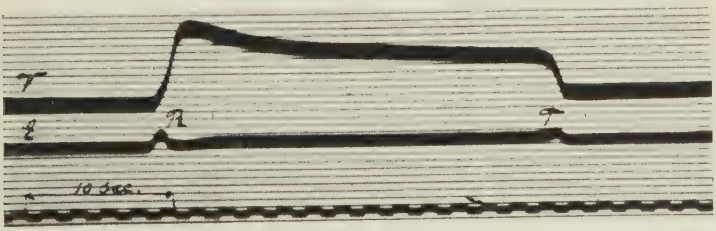

Fig. 16.—Same conditions as in Fig. 15 with continued poisoning. *v. Lawick v. Pabst.*

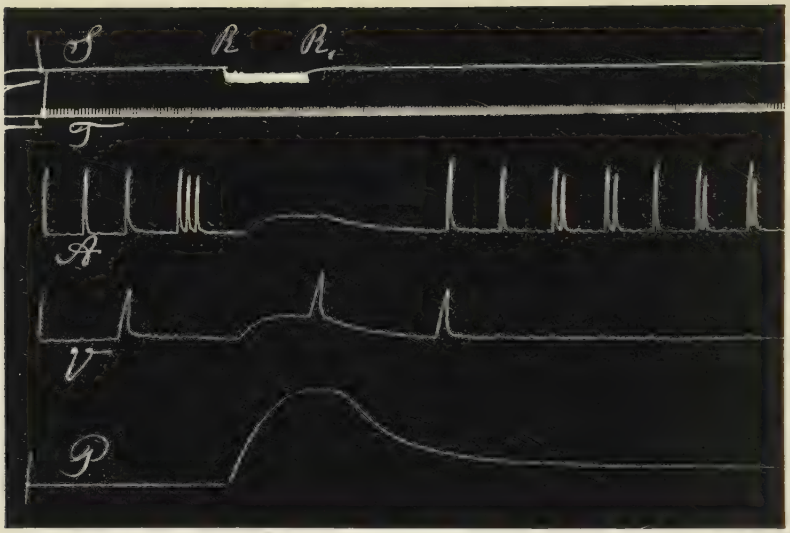


Fig. 17.—Tortoise. *S*, signal; *T*, time-axis, the distance between two vertical lines corresponds to one sec. *A*, mechanogram of the right auricle; *V*, mechanogram of the ventricle; *P*, pneumogram. Between *R* and $R_1$ the vagus is stimulated. *Rademaker.*

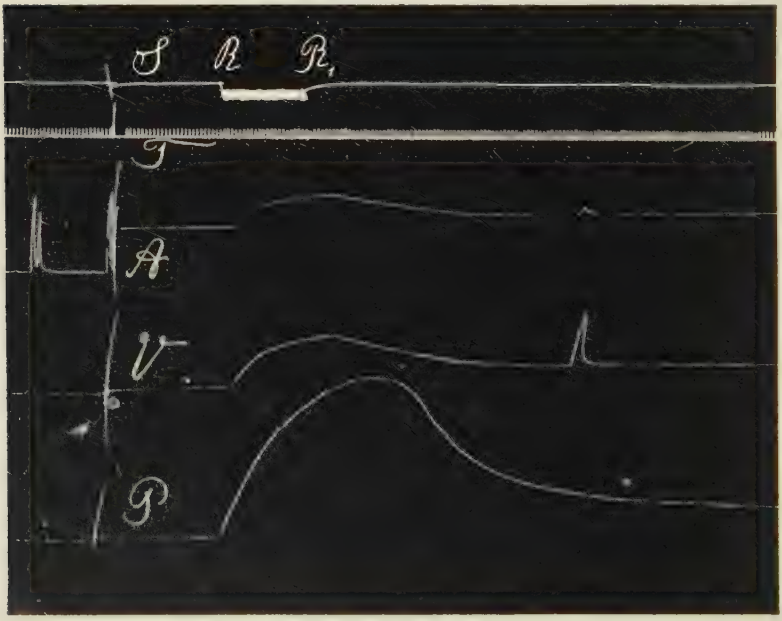


Fig. 18.—Similar conditions as in the previous figure. The heart had come to a standstill. *Rademaker.*

We may now proceed to the discussion of Gaskell's well-known experiments. They were carried out in the following way. The heart of a tortoise is prepared in such a manner that the auricles are separated by a cut from the sinus and brought to a standstill. The apex of the right auricle is drawn up a little and injured. From the injured apex and from the uninjured auricular tissue near its base the demarcation-current is led off to the galvanometer. Care is taken that in separating auricle and sinus the vagus nerve remains intact. Thus with a stimulation of this nerve its effect on the heart might still be expected.

Now what did Gaskell observe? That with a stimulation of the vagus the demarcation-current increased and thus in contrast with any skeletal-muscle, whose nerve is stimulated, showed a positive fluctuation. This experiment, described by *Gaskell* in 1887, has had a great influence and has helped to establish the theory, that the electric current would be the expression in the one case of a wave of excitation corresponding to a katabolic effect, in the other of a wave of inhibition corresponding to an anabolic effect; and that the contraction was to be looked upon as secondary, something independent of the electric current.

A closer examination, however, shows that this conclusion cannot be correct. The cause of the positive fluctuation of the current is to be found in a movement of the auricle. When the peripheral end of the vagus is stimulated, the lungs of the tortoise contract. In Gaskell's preparation the heart rests on the lungs, and when these contract the heart drops backwards with them. The right auricle, which is fastened to a hook by a thread, is thereby somewhat extended, and this extension is the cause of the current-fluctuation.

We shall try by means of a few curves to demonstrate, first, that when the lungs contract on excitation of the vagus the auricle is actually extended; in the second place, this extension of the auricle leads to positive variation of the demarcation-current.

In figure 17 $S$ represents the signal; $T$ is the time-axis.

The distance between two vertical lines here has the value of one second. A lever is attached to the apex of the right auricle and describes line $A$; a second lever registers the movements of

the ventricle *V* and a third those of the lungs *P*. The preparation is no longer fresh, the heart has suffered much, and the auricle and ventricle only beat in an irregular manner. There is, moreover, a block between auricle and ventricle. Between R and $R_I$ the vagus is stimulated during about 25 seconds. The effect on the lungs is seen, and at the same time that on the heart. The contraction of the auricles has been stopped, but owing to the block the ventricles go on beating.

The next figure 18 gives a confirmation of the previous one. In this case we have a preparation comparable to that of Gaskell. The heart has come to a standstill, and when the vagus nerve is now stimulated we see the curves of the auricle, ventricle and lungs ascend simultaneously. The contraction of the lungs in the tortoise is very slow. It begins after a latent period of about 3 to 4 seconds, reaches its maximum in about half a minute and is not finished till after three or four minutes. This exceptional slowness has probably been the cause of the contraction not having been noticed previously.

We now show the curves which prove that an extension of the auricle occasions a positive variation of the demarcation-current.

In figure 19 *L* is the pneumogram, *E* the electrogram of the right auricle, the electro-atriogram. Between R and $R_I$ the peripheral end of the vagus is stimulated. A remarkable correspondence between the contraction of the lung and the electro-atriogram will be seen here. Even small oscillations, displayed by the contraction of the lung, are similarly found in the galvonometric curve. It is true that the similarity is not an absolute one, but we cannot expect that, in comparing two phenomena, the one mechanical and the other electrical. As it is, the similarity is, indeed, sufficiently striking.

In the next figure 20 we have a heart which has been poisoned with muscarin and thus brought to a standstill. The right auricle is again injured, in the same way as in the previous experiment, and the current is also collected from the auricle to the galvanometer in the same manner. Again the corre-

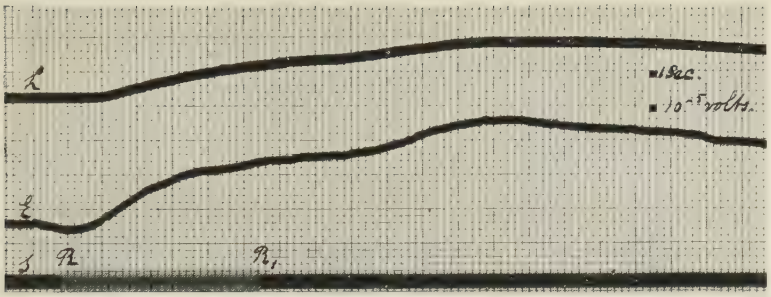


FIG. 19.—Tortoise, *L*, pneumogram; *E*, electrogram of the right auricle. Between *R* and $R_1$ vagus stimulation. *Rademaker.*

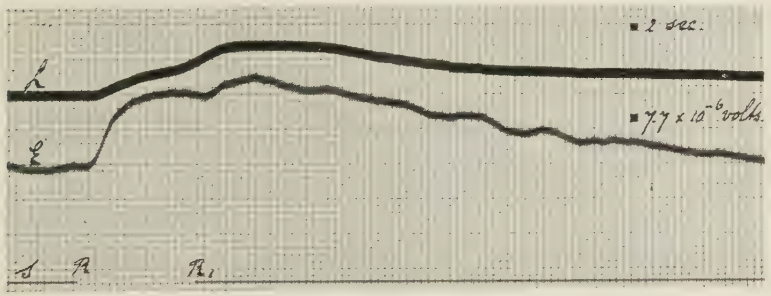


FIG. 20.—Tortoise poisoned with muscarin. Similar conditions as in the previous figure. *Rademaker.*

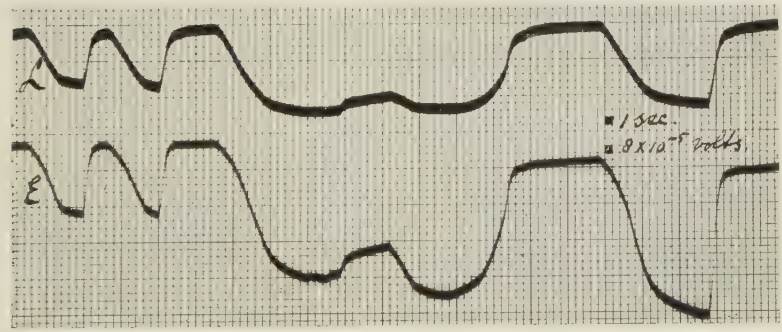


FIG. 21.—Tortoise. *L*, pneumogram; *E*, electro-atriogram. The lungs are alternately inflated and slightly deflated. *Rademaker.*

spondence between pneumogram and the electrical phenomenon is striking.

In the next figure 21 we did not stimulate the vagus, but the lungs were alternately inflated and slightly deflated. By the movement thus produced, the right auricle was necessarily extended more or less and we notice again a correspondence between the variations in the volume of the lungs and the current fluctuations of the right auricle. Purposely the inflating and deflating of the lungs was made to take place irregularly. That all these irregularities are also found in the electro-atriogram may be looked upon as a definite proof that the current fluctuations in the auricle are caused by increase or diminution of the extension of this organ. Gaskell's positive current variation is not a vital process, not an expression of the inhibitory action of the vagus, but a physical process, a physical effect of the extension of the organ.

The curves shown here prove more than the incorrectness of Gaskell's interpretation. They allow the drawing of a positive conclusion. The inhibition of a heart's action by the vagus is a phenomenon studied by all physiologists. There probably is not a physiological laboratory where this vagus-experiment has not been made. The proof is repeated annually in hundreds of courses of medicine or physiology as being equally easily demonstrated as instructive to the audiences. If in an inhibition experiment so clear and easily controlled a *positive* current-variation cannot be demonstrated, we have the right to assume, that neither is the *negative* current fluctuation of a striated muscle caused by the excitation-wave, but is closely connected with the contraction itself.

Finally, we shall discuss the experiments which have made the deepest impression on investigators, and which seemed to be the most conclusive towards proving that the mechanical and electrical phenomena may be separated. They have been described by various experimenters from different countries. It is said that a heart may be brought to a mechanic standstill by injury, removal of indispensable constituents and also by the action of various drugs, while the rhythmic electrical variations

continue. Here we have an instance in which the mechano-cardiogram ceases, whereas the electro-cardiogram continues to be recorded undisturbed. A more striking experiment to demonstrate the separability of the two processes can hardly be conceived.

These experiments have reference to the frog's heart; they are easily made, and in the hands of the various investigators have so far yielded corresponding results. There is no denying the fact itself that the marking lever of an ordinary registering apparatus may stop, while the galvanometer quietly continues to record the electro-cardiogram. But the conclusion drawn from this fact, that the mechanical and electrical processes are actually separable, is incorrect. This beautiful and seemingly so conclusive proof is not a proof at all, and the phenomenon only arises through the imperfection of the instruments used and the inadequacy of the methods adopted.

In the first place, attention must be drawn to the vast difference in sensitiveness between the mechanical and the electrical measuring instruments, as universally used. Thus, for instance, the string-galvanometer is many hundred-thousand times more sensitive than the ordinary mechanical registering arrangement. It can register an electric current, the energy of which corresponds to that which is required to lift 1 mm.$^3$ of the heart to a height of $10^{-11}$ mm. If the weight of a frog's heart is put at 100 mg., we may also say that the energy required to produce a visible deviation of the string corresponds to that of the lifting of one-ten-thousandth part of the heart to a level of one-millionth of a micron. The mechanical recording contrivance does not exist which would approach anything like this sensitiveness. This fact must not be lost sight of when we wish to compare the results.

The second remark which we wish to make has reference to the friction. A mechanical registering apparatus, as a rule, has to overcome the mutual friction of solid parts. Unless the registering is done photographically, the marker travels with its point over the writing surface, neither does the axle of a rotating lever move without friction in its bearings, whereas in the elec-

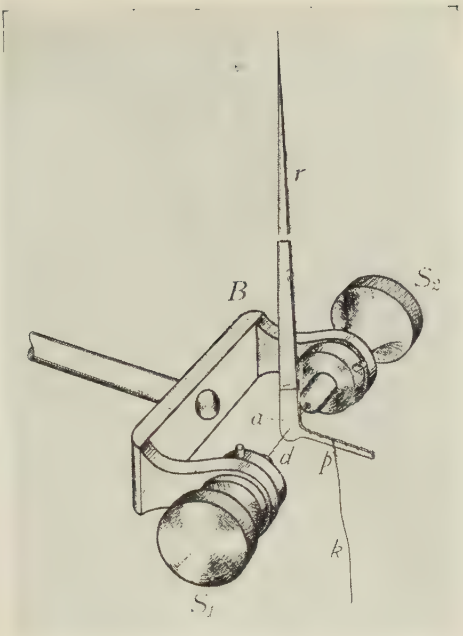


FIG. 22.—String recorder. Vertical motions of the cord $k$ produce horizontal ones of the pointer $r$.

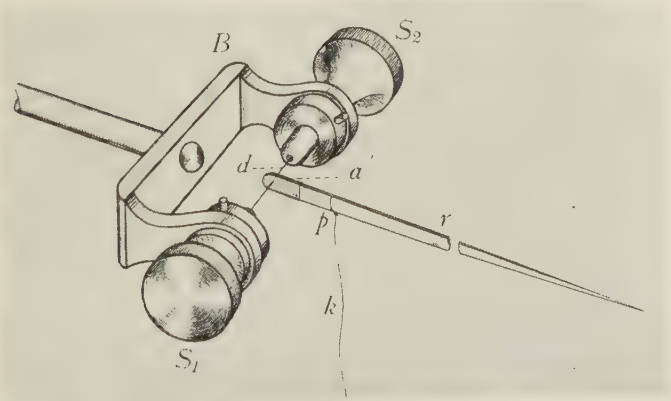


FIG. 23.—Similar apparatus as in Fig. 22. The pointer $r$ and the cord $k$ move in the same direction.

trical registration there is only some air-friction to be overcome. There is this great difference between the friction of solid parts, on the one hand, and air-friction on the other; the latter approaches zero, as the velocity of motion becomes very small or disappears. On the contrary, solid friction increases as the motion becomes slower and approaches a maximum as the motion reduces to nothing.

In the third place we draw attention to the fact which may lead to a false conclusion and is connected with the heart itself. If a heart is brought to a standstill by poisoning it with digitalis, when it will be in a condition of strong systole, or if it is injured by pouring paraffine over it in order thus to bring it to a mechanical standstill, it remains possible that differences in tension develop in it, which may produce mutual displacement of the separate fibres, but are unable to displace the external surface of the heart and thus impart motion to a lever. Under these circumstances there are isometric contractions in the heart which can produce an electrogram, but not a mechanogram.

When the heart is perfused, the friction of the perfusing solution in the heart will also have to be taken into account. This friction is not sufficiently removed by cutting the two aortic arches or even opening the bulb, especially if the severed parts of the wall should come in contact again and stick together. When the heart is very much weakened, we again meet with a condition that external movements are absent, while in the inside forces are acting which are associated with the development of electric currents.

When all these difficulties are avoided, totally different results are obtained.

For the registration of the mechanogram, we have used a recording arrangement acting without friction. To a tightly drawn metal wire $d$ a lever is soldered. The long arm of the lever is a pointer which registers the motions photographically; the short arm is attached by a cord to a point of the heart's ventricle at $k$. At $S_1$ and $S_2$ we have the screw heads by means of which the torsion of $d$ may be increased or diminished. This metal wire may be considered as perfectly elastic. Each move-

ment of the heart will be thus transmitted without the least chance of friction. We may call this piece of apparatus a "string-recorder" and I may mention that it is very similar to that constructed by Mines.

The recorder of figure 22 registers vertical motions in such a manner that they are changed to horizontal ones. It is, of course, also possible to design the apparatus so that vertical motions remain vertical, as shown in figure 23. We shall now describe a number of records obtained.

In figure 24 are seen the curves obtained when the heart's action is registered in a case of KCl poisoning. The current is taken from the apex and the base of the heart. Previously the apex has been injured, with the object that with each contraction the same regular form of electrocardiogram may be registered. If the heart had been left uninjured, it would have been possible for the contraction-wave to change its direction in the course of the experiment. The form of the Ecg would thereby become variable while the mechanogram remained practically unchanged. Here is another instance of dissimilarity between electrogram and mechanogram, although it does not in the least prove that the two phenomena are not most closely connected. To avoid this we have always injured the apex.

*A* is the curve of the auricular, *V* that of the ventricular movement, *E* the electrogram of the ventricle.

In the first part of the figure before the beginning of the poisoning we notice large deflections of both mechano- and electrogram, which on the application to the heart of drops of KCl-solution diminish in height, almost proportionally, until both groups of waves in the last part of the figure become so small that they can hardly be perceived.

The opinion prevailed, that by means of the poisonous KCl it was possible to demonstrate the mutual independence of the electro- and mechanogram quite easily. But the curves show that if the experiments are made with sufficient accuracy, both groups of phenomena are about equally susceptible to the action of the poison and remain parallel to the end.

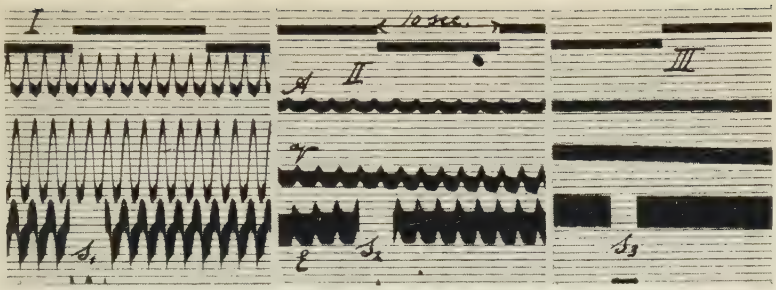


Fig. 24.—Frog. I, before poisoning. II, poisoned by means of a KCl solution. III, poisoning continued. *A*, Mechanogram of the auricle, *V*, mechanogram of ventricle, *E*, ventricular complex of the electrocardiogram. At $S_1$, $S_2$, $S_3$, the sensitiveness of the galvanometer is tested by introducing $10^{-2}$ volts in the circuit. *Arbeiter.*

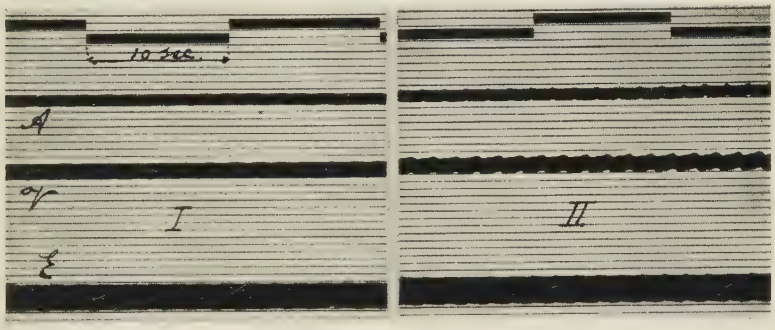


Fig. 25.—Frog's heart. I, after being perfused for 24 hours with a Ca-free Ringer-solution. II, Gradual increase of the mechanical and electrical phenomena on the addition of lime-salt. *A*, mechanogram of the auricle, *V*, of the ventricle, *E*, Ecg. *Arbeiter.*

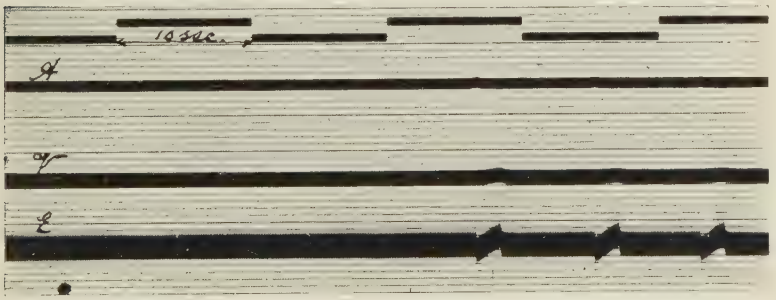


Fig. 26.—Frog's heart. Standstill produced by perfusion with a Ca-free Ringer-solution. Sudden recovery on addition of Ca. Simultaneous reappearance of the mechano- and the electrogram. *Arbeiter.*

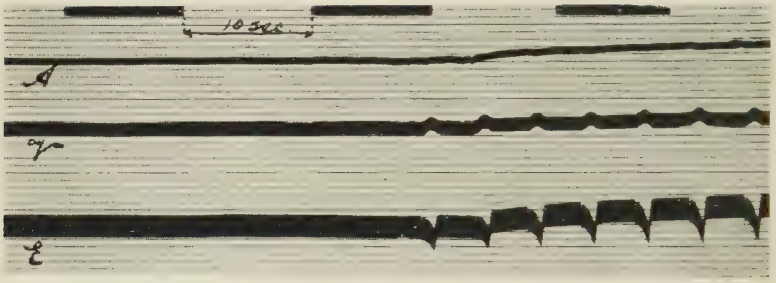


Fig. 27. —Another instance of sudden recovery and simultaneous reappearance of the two groups of phenomena. *Arbeiter.*

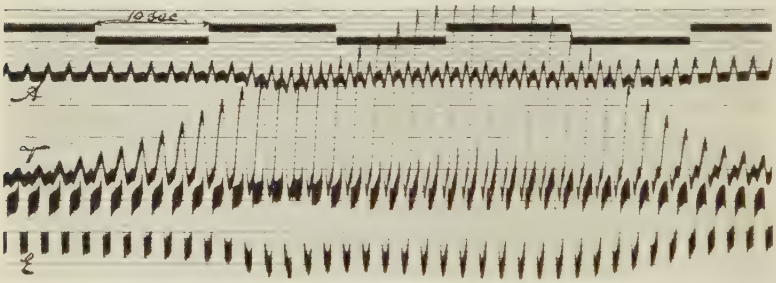


Fig. 28.—Addition and elimination of Ca. *Arbeiter*

On the other hand, it is quite simple to arrange the experiment in such a manner that the mechanogram disappears before the Eg. We have only to make the marker of the mechanical heart-action work with some friction.

Another means of separating the two groups of phenomena consisted in the removal of calcium from the heart. The heart was perfused with a Ringer-solution, from which the calcium had been removed. Soon the heart was then found to come to a mechanical standstill.

Against this we have found that even after being perfused for 24 hours with a Ca-free Ringer-solution the heart does not cease to contract. It is true that after a relatively short time—about half an hour—the power of the heart's contraction is very considerably diminished, and this circumstance has probably induced investigators to assume that the heart has stopped. But if the recording apparatus is sufficiently sensitive, one can even after 24 hours continue to register the regular rhythmic contractions, while the electrical deflections always run parallel to the mechanical action.

In figure 25 a curve is seen in which the mechanical and electrical actions are both very weak, but still distinctly visible, after the heart had been perfused for 24 hours with a solution without a trace of lime salt. On the addition of Ca a slow and gradual return of both groups of phenomena took place, as is shown in the second part of the curve.

Sometimes the heart recovers suddenly, when fresh calcium is added, but always a simultaneous reappearance of the two oscillations will be noticed, comp. figures 26 and 27.

In figure 28 we have a curve in which, after removal of calcium followed by its replacement a diminution of the waves in the mechano- and electrogram can be noticed followed by an increase. The proportionality is not so close as might be expected, but we had omitted in this case to remove the friction in the heart as much as possible. For that purpose one must cut open the apex of the heart completely, in order that the solution may be able to flow off freely. Otherwise, the friction of the liquid which has to flow off through the bulb and the two aortic

arches has an influence which cannot wholly be neglected. If the experiment is made as it should be, one finds proportionalities which are wholly satisfactory, when the amplitude of the oscillations of the two curves is reduced in a ratio up to 500 : 1.

In figure 29 we see a case of poisoning by chloroform vapor, in figure 30 a perfusion with a Ringer charged with chloroform, in figure 31 a case of poisoning by curari, followed by the application of atropin.

All our experiments with poisoning, injury or removal of indispensable constituents have given us the same result, namely, a complete parallelism of the mechanical and electrical phenomena, always changing pari passu. This is the uniform result, when an appropriate method is applied, and the instruments used are sufficiently sensitive.

I have reached the end of this demonstration and discussion. In conclusion I may be allowed to make a few general remarks, with regard to the bearing of the subject treated. This bearing is two-fold.

In the first place, it is theoretical. It may help us to study the relation of metabolism in the muscle, heat-production, the generation of electric currents and the production of mechanical forces.

In the second place, the experiments are of practical significance. If it is once known that the electric variations are, indeed, intimately connected with the contraction itself, we may make inferences regarding the action of muscles from the deflections of the galvanometer in all those cases in which we are unable to make direct records of their contractions.

The electrocardiogram may be quoted as an example. From the curve traced by the galvanometer we do not only draw conclusions as to the path of conduction of the excitation wave, but also as to the mood of the heart's contraction. The medical practitioner thus stands on firm ground, when building on the electrocardiogram, and we may see in this a new instance of how purely theoretical problems may ultimately turn out to be of practical use.

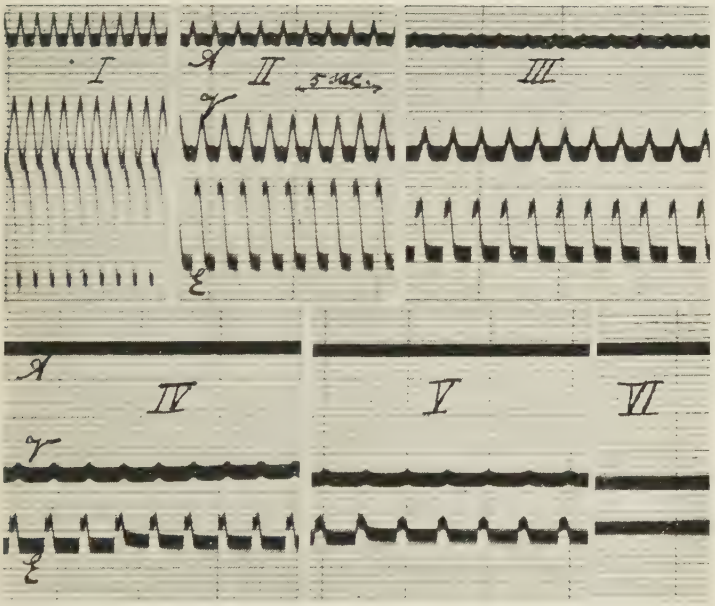


FIG. 29.—Poisoning with chloroform vapor. I, before application of chloroform. In II, III, IV, V and VI gradually more and more chloroform is applied. At the end of the experiment the mechanical as well as the electrical phenomena stop simultaneously. *A*, *V* and *E* have the same signification as in the previous figures. *Hugenholtz.*

Fig. 30.—Frog's heart perfused with a Ringer-solution charged with chloroform. *Hugenholtz.*

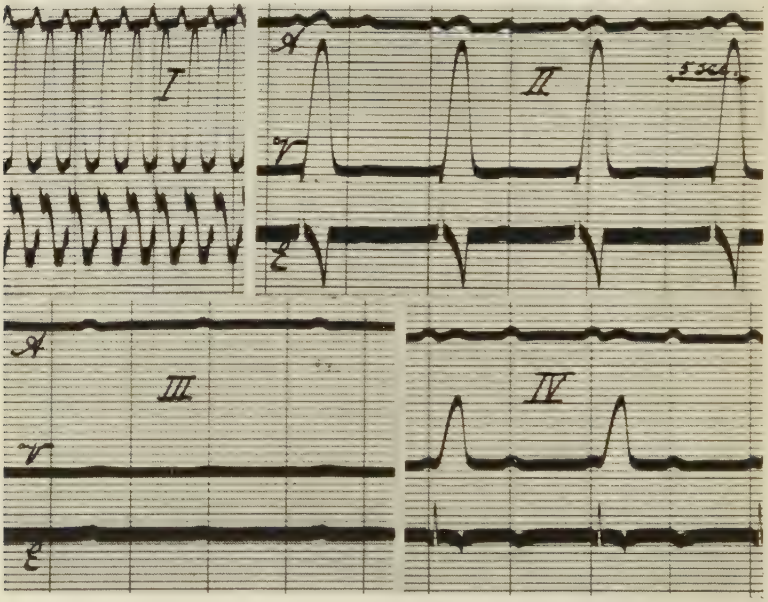


Fig. 31.—Frog's heart. I, normal. In II and III the effect of large doses of curari is seen. In IV the effect of curari is partly abolished by the application of atropin. The electrical and mechanical phenomena show a complete parallelism. *Hugenholtz.*

# ETIOLOGY OF SCARLET FEVER *

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SCARLET FEVER is in all probability a very old disease. The regions in which the malady originally arose are a matter of uncertainty. There are some who believe that the Plague at Athens was a malignant form of scarlet fever, an interesting assumption in view of the present relatively low case fatality and the ominous variability in severity of outbreaks in the past. Fairly accurate descriptive records of scarlet fever appear in the literature as early as the middle of the sixteenth century and recur with increasing frequency and definiteness up to the time of Sydenham. For many years the disease was confused with measles, erysipelas, diphtheria and certain septic processes. Sydenham, who first employed the name scarlet fever, clearly differentiated it from measles by his careful description of the disease as it appeared in London from 1661 to 1675, and laid the foundation of an accurate knowledge of its special characters. In spite of this valuable contribution, the existing confusion did not disappear, and many physicians still confounded it with diphtheria and certain septic anginas. With the increasing volume of medical literature and better facilities for the communication of ideas, scarlet fever became more and more clearly defined as a clinical entity. Confusion, however, with diphtheria frequently occurred, even down to the times of accurate diagnosis by means of bacteriological methods. In fact, even today the inability to differentiate scarlet fever from certain septic conditions of the throat, associated with erythematous rashes, continues to plague the mind of the diagnostician. In spite of these diagnostic difficulties clinical differentiation of scarlet fever from other exanthemata has been possible for a long enough

* Lecture delivered January 17, 1925.

period of time to determine clearly its contagious nature and to permit illuminating epidemiological and clinical studies.

The contagious element in scarlet fever is probably always derived from a previous case. In most instances it is taken directly into the mouth or nasopharynx by the inhalation of air charged with minute droplets of saliva or mucous projected from the mouth or nose of the infected individual. Other important sources of contagion are the purulent discharges from infected paranasal sinuses, from suppurative inflammation of the middle ear and lymph glands, secondary conditions that constitute the most frequent and distressing complications of the disease. There is much evidence to support the view that the causative organism survives in the dry state in a virulent form for long periods of time. Contamination, therefore, of clothing or personal articles of any kind with infective matter may serve as a means of conveying scarlet fever. Formerly the belief was quite prevalent that flakes of skin given off during the period of desquamation were the most important vehicle of the contagion, and quarantine regulations were roughly founded on time periods corresponding with the duration of the desquamative stage. Current opinion holds that the contagious element is not present in the skin in the late stages of scarlet fever, a somewhat curious fact, inasmuch as the rash is the most distinctive clinical manifestation of the disease. The rôle of the healthy carrier in spreading scarlatina is undoubtedly of great importance but determined accurately as yet in only a very few instances because of the uncertainty concerning the etiological agent. That such types of carriers do exist there can be no doubt, and Bliss [1] has been able to trace a small epidemic of scarlet fever to such a source. Another interesting means of the wide dissemination of scarlet fever is an infected milk supply and numerous undoubted outbreaks have arisen from the consumption of contaminated milk. The clinical and epidemiological evidence, therefore, that has been collected indicates that the causative agent of scarlet fever is present in the throat secretions and the discharges from suppurative foci in patients throughout the illness, and for a considerable period of time during con-

valescence. It resists exposure to light, and in the dry state may retain its infectivity for many months. Healthy carriers and atypical attacks of the disease are not an infrequent occurrence. In all probability the udder of the cow may become infected with the specific virus, and the milk obtained from this animal may serve as a vehicle of infection.

Notwithstanding these excellent clinical and epidemiological studies which have ensured the easy recognition of typical attacks of the disease, and which have furnished the essential data for useful quarantine regulations, the causative organism of scarlet fever has remained unknown. Experimental studies have been published from time to time, suggesting that the infective agent belongs to one or other of the principal groups of microörganisms, such as the pathogenic bacteria, protozoa, and the so-called ultramicroscopic viruses. As a bacterial cause, Streptococcus hæmolyticus has aroused much interest and stimulated more or less continuous investigation because of its constant association both with the uncomplicated and complicated forms of the disease. Certain observers have discovered inclusion bodies in leucocytes and in epidermal cells which they have thought indicative of a protozoan cause for scarlet fever. Finally, scientific opinion seized upon those mysterious living bodies commonly designated as filterable viruses as the most probable cause of the disease. This latter view has become most widely accepted and is the usual etiology assigned in text books in spite of the fact that no real evidence has ever been produced to show that any such microörganism exists either in the throat secretions, tissues or blood of an individual suffering from scarlatina.

Both Mallory [2] and Döhlé [3] have made the suggestion that scarlet fever may be due to a protozoan infection. In 1904 Mallory observed in four cases of scarlet fever certain bodies whose varying morphology strongly suggested that they might have been stages in the development cycle of a protozoan. They occurred in and between the epithelial cells of the epidermis and free in the superficial lymph vessels and spaces of the corium. They formed a series of bodies including definite rosettes, which

closely resembled those seen in the asexual development of the malarial parasite. There were also certain coarsely reticulated forms which he thought might represent stages in sporogony. Mallory was of the opinion personally that these bodies were protozoa and bore an etiological relationship to scarlet fever, but he did not regard their significance as established. Confirmatory observations were subsequently made by Duval,[4] Bernhardt[5] and v. Prowaçek.[6] Similar bodies, however, were later found by Field[7] in other conditions and they finally came to be looked upon as peculiar products of cell degeneration and not as living forms with a specific relationship to scarlet fever.

In 1912 Döhlé, on examining the blood smears from thirty cases of scarlet fever, found within the cytoplasm of the neutrophilic polynuclear leucocytes multiform inclusion bodies. These inclusions were present in a large percentage of all leucocytes and by special methods of staining revealed themselves as intermediate in intensity between nucleus and cytoplasm. In a later communication certain of these inclusions are designated as "Spirochæta scarlatinæ," and are assigned both diagnostic and prognostic importance. Although numerous observers confirmed Döhlé's observations on the presence of leucocytic inclusion bodies, further study revealed the fact that they are present in practically all febrile conditions, in chronic pyogenic infections without fever, in certain severe injuries, and occasionally in normal human beings. In all likelihood they result from nuclear degeneration not infrequently observed in septic states and have no specific bearing on the etiology of scarlatina. The evidence offered in favor of the protozoan origin of scarlet fever has never stood the test of close scrutiny.

The belief that scarlet fever is due to an unknown virus, probably of filtrable character, is based largely upon the results of attempts to communicate the disease experimentally to animals. A number of observers have reported scarlatina-like manifestations in monkeys inoculated with infective material from active human cases of the disease. Among these observations the most interesting are those of Levaditi, Landsteiner and Prasek,[8] who, by the inoculation of anthropoid apes, seem to have pro-

duced what in all likelihood was true scarlatina. Exudate from the throats of individuals with scarlet fever was rubbed into the tonsils of apes and defibrinated blood injected subcutaneously, and in one instance material from a suppurating lymph gland. The animals, after an incubation period of about three days, are described as having a typical angina with characteristic exudate, enlargement of the follicles of the tongue, a generalized exanthem resembling that of scarlet fever, and in certain instances when the animals recovered desquamation of the skin. There was also present the characteristic lymphoid hyperplasia, and the histological lesions in the skin resembled those seen in scarlet fever. In all the animals presenting such a picture S. hæmolyticus was present, either in the blood or in the local lesions in the throat. Levaditi, Landsteiner and Prasek, however, did not think that streptococcus was accountable for the manifestations, inasmuch as when pure cultures of this organism were obtained from the infected animals or from human beings and inoculated into fresh apes, the phenomenon described could not be reproduced. They do not state that the organism of scarlet fever is a filtrable virus, but simply say that it is of unknown characteristics. Cantacuzene [9] and Bernhardt [10] claim to have induced a similar series of phenomena by the inoculation of monkeys of a lower order with human material. Levaditi, Landsteiner and Prasek failed to produce in a large series of lower monkeys the disease syndrome manifested by the apes, nor were subsequent investigators more successful in confirming the observations of Cantacuzene and of Bernhardt. From the failure to discover an organism of known characteristics, rather than from any positive evidence has grown the belief so generally held that the etiological agent of scarlatina is an ultramicroscopic virus.

During the many years that investigators have searched for the causative agent of scarlet fever, and with the varying emphasis attached to one or another species of parasite from time to time, the constant relationship to this disease of one organism, S. hæmolyticus has become more and more significant. As early as 1885 Crook [11] reported the presence of streptococcus in the blood and organs of individuals dying of scarlet fever.

Loeffler,[12] in addition, found this organism to be present in certain types of necrotic angina associated with scarlet fever and was furthermore successful in isolating the germ in pure culture. At this time Klein [13] likewise isolated a streptococcus from the tissues of patients with scarlatina, which he named Streptococcus scarlatinæ. In 1885 the latter observer, while investigating an outbreak of fever among certain cows belonging to a farm at Hendon, England, isolated from ulcerative lesions of the udders and from certain viscera, a streptococcus which he considered to be identical with Streptococcus scarlatinæ. This observation was not only of great interest but also of very great importance, because the milk obtained from the infected cows was shown to have been consumed by persons who subsequently developed scarlet fever. These early observations of the frequent relationship of streptococcus to scarlatina were soon confirmed by many students of the disease in different parts of the world. In 1900 Baginsky and Sommerfeld [14] reported the constant presence of streptococcus in the throat during the characteristic angina in seven hundred cases of scarlet fever. They also found this organism frequently in the blood, bone marrow and internal organs of patients dying of this disease. Other observers found streptococcus in the blood of fatal cases of scarlet fever in as many as 70 per cent. of the individuals studied. Hektoen,[15] furthermore, found the organism in the blood in 12 per cent. of patients during life, and his observations are of especial interest in that they indicate that the usual bad prognostic import of this phenomenon does not necessarily hold for scarlet fever.

In addition to the presence of streptococcus in the throat and blood of individuals with scarlet fever, this organism has also been proven to be the most frequent cause of the septic complications of the disease. Many times in septic foci streptococcus has been found in pure culture, and it is an old observation that convalescent individuals with discharging suppurative lesions are especially likely to give rise to return cases of scarlet fever, showing that in such lesions the causative virus persists in an active form for long periods of time.

Such widespread and constant association of S. hæmolyticus

with scarlet fever has led some investigators to propose the view that streptococcus is the etiological agent of this disease. Certain observers, on the other hand, oppose this belief and have considered it more likely that streptococcus plays in scarlatina the rôle of a secondary invader. The objections of this latter group to the etiological significance of streptococcus are based upon certain important considerations. As is well known, streptococcus is an organism of very widespread distribution and gives rise to a variety of pathological lesions, such as abscess formation, cellulitis, septicæmia and numerous other conditions. Frequently the same individual may have throughout life repeated streptococcus infections, one attack not seeming to confer immunity against subsequent invasion of the tissues by the same organism. The latter condition of affairs is especially true of erysipelas, one of the most characteristic of the streptococcus diseases. On the other hand, scarlet fever, in sharp contrast to other streptococcus infections, is a fairly definite clinical entity and one attack appears to give rise to an immunity of life-long duration. This peculiarity of scarlet fever might have been explained had it been possible to prove that the streptococcus associated with scarlet fever differed specifically from the hæmolytic streptococci causing the various septic processes. However, early cultural and biochemical studies have failed to demonstrate any significant differential characteristics by means of which Streptococcus scarlatinæ could be separated biologically from similar streptococci found in other diseases. When grown in fluid or in solid media, hæmolytic streptococci resemble one another very closely, whatever be their source. It is true that certain constant differences can be brought out by means of fermentation of various carbohydrates, but such variations as exist apparently do not bear any specific relationship to a single disease process, and have been of but little aid in determining the etiological significance of streptococcus in scarlet fever. In addition to this objection, Jochmann [16] has emphasized especially his failure to find streptococcus in either blood or tissue of individuals dying in a few days from malignant forms of the disease. Since, therefore, types of streptococci indistinguishable from

those seen in scarlatina are found in a great variety of disease conditions, and since the quality of the immunity in this disease differs widely in its duration from that observed in other streptococcus infections, and finally because of Jochmann's contention that streptococcus is not present in certain malignant types of scarlet fever, the conclusion has been drawn that streptococcus cannot be the cause of the disease.

An effort to meet these objections has been made by the group of investigators who believe that streptococcus is the etiological agent of scarlet fever. The observation by Baginsky and Sommerfeld of the constant presence of S. hæmolyticus in the throats of all cases of scarlatina, an observation later confirmed by others, has done much to offset the inferences drawn from Jochmann's failure to find it in a few instances of fulminant types, especially since we now know that the organism in the latter cases may have been localized in some inaccessible area. Attempts were made in addition to explain the immunity in scarlet fever and to establish the type specificity of the scarlatinal streptococcus. Moser [17] and Moser and Pirquet [18] have claimed that scarlatinal convalescent serum agglutinates to a higher titer Streptococcus scarlatinæ than does control serum from other diseases. Furthermore, they have prepared polyvalent serum from horses, using the streptococcus of scarlet fever as antigen and have studied the capacity of such sera to agglutinate specifically various strains of scarlatinal streptococci. The latter strains were agglutinated in dilutions of 1 : 1000 or over, whereas hæmolytic streptococci from other sources were not specifically agglutinated. As a consequence of these observations Moser and Pirquet believed that the streptococcus of scarlet fever differs specifically from apparently similar strains isolated from instances of erysipelas, phlegmon and puerperal sepsis. Meyer [19] and Rossiwall and Schick [20] have confirmed the results of Moser and Pirquet. Unfortunately, however, certain later studies by Hasenknopf and Salge,[21] Aronson,[22] and Neufeld [23] failed to support the earlier ones and grave doubt was thrown upon the specificity of Streptococcus scarlatinæ.

Other interesting facts which indicate the specific relation-

ship of streptococcus to scarlet fever have come from the studies of Gabritchewsky[24] on the specific prophylaxis of scarlet fever by means of a vaccine prepared from S. scarlatinæ, and of Moser[25] on the therapeusis of the disease by means of a specific antistreptococcus serum. Gabritchewsky and his co-workers immunized a large number of individuals against scarlet fever with a vaccine prepared from hæmolytic streptococci isolated from scarlatina. During the process of immunization certain phenomena occurred which were highly suggestive of the clinical manifestations of scarlet fever. In the majority of instances an area of erythema and swelling averaging 15 cm. in diameter developed at the site of the vaccine injection appearing in from eight to twenty-four hours and lasting about forty-eight hours. In general, the erythema was diminished or absent following a second injection some ten days later. In about 15 per cent. of the individuals inoculated a general reaction was observed. This general reaction consisted in fever of 1° C. or so, leucocytosis and an erythematous rash, having the characteristic distribution of the exanthem in scarlet fever. Some of those inoculated showed the typical angina and strawberry tongue peculiar to the disease and in a few instances signs of renal irritation were observed. In general individuals who were recovering from the disease or who had had it some years before failed to show either a local or general reaction. Administration of Moser's antiscarlatinal serum before the inoculation was shown to prevent the development of both a local and a general reaction. Prophylactic immunization of this type seemed to diminish the incidence of scarlet fever among the inoculated. As a result of these observations Gabritchewsky and his assistants were strongly of the opinion that streptococcus is the causative agent of scarlet fever.

The therapeutic results obtained by the use of Moser's serum lent further support to this view. Moser immunized horses to hæmolytic streptococci obtained from the blood of patients suffering from scarlatina. The serum thus prepared was used therapeutically and is said to have had marked beneficial effects causing a drop in the temperature and pulse, a diminution of the toxæmia, early disappearance of the rash and a marked short-

ening of the duration of the disease. Escherich, who observed the work closely, was much impressed by the therapeutic value of the serum and likened its action to that of diphtheria antitoxin. Later antistreptococcic sera prepared by other investigators, however, failed to display the therapeutic efficiency of Moser's serum and created doubt in the minds of many concerning the usefulness of such sera.

Much other evidence both for and against the etiological relationship of streptococcus to scarlet fever was presented at this time and as one weighs its importance in retrospect, the positive seems of more significance than the negative. The outstanding objection, however, to the acceptance of streptococcus as the cause of scarlet fever remained the impossibility of differentiating satisfactorily this organism from hæmolytic streptococci associated with the great variety of septic conditions. As a result, other etiologic agents were searched for. Moser's serum dropped into disuse and streptococcus vaccine was no longer used in the prophylaxis of scarlet fever. Scientific opinion gradually came to hold that streptococcus bore an important but secondary relationship to scarlet fever, and that the true cause must be sought among the unknown viruses.

For many years confusion has existed and opinion has varied concerning the existence of biologically varying types of streptococcus. Two diverging points of view developed, one maintaining the unity of the species as a type, and the other holding that it comprised a group of organisms different from one another in their biological characteristics. Schottmüller [26] in 1903 made an important contribution to the discussion in demonstrating between certain streptococci, differences based on their action on blood agar plates, one group hæmolyzing the red blood cells and the other either failing to hæmolyze or forming methemoglobin. This significant differentiation resulted in the establishment of the types now generally recognized as hæmolytic and nonhæmolytic or green pigment producing strains. Further classification was attempted by numerous investigators who used as a basis of differentiation certain biochemical reactions. Holman [27] in 1916, using carbohydrate fermentation as a

test, was able to demonstrate the existence of a number of separate fermentation types. Numerous efforts were also made to establish biological differences, especially among the hæmolytic streptococci, by means of serological reaction methods which had proven singularly successful when employed for studying the various types of pneumococcus and meningococcus. As a result of these studies conflicting beliefs arose, and a definite opinion could not be given as to whether or not separate biological types of S. hæmolyticus exist. As late as 1918 Swift and Kinsella,[28] using the complement fixation reaction as a test, made a series of observations of twenty-eight strains of hæmolytic streptococcus from various sources. They found that they were unable to determine significant serological differences between the strains studied and are of the opinion that a striking homogeneity exists. Efforts to correlate such different types of hæmolytic streptococcus as had been determined with specific pathological lesions were also of indeterminable significance, varying types being found in association with the same disease.

In 1918 Dochez, Avery, and Lancefield[29] undertook a biological study of a great number of strains of S. hæmolyticus, obtained from a variety of pathological conditions among the changing population of a large military establishment. The purpose of this investigation was to determine if there exist among the hæmolytic streptococci diverse biological types, as is the case in the instances of pneumococcus and meningococcus. The specific test reactions were those of agglutination and protection. Spontaneous non-specific flocculation, the most confusing factor in previous studies of specific agglutination of streptococcus, was avoided by the employment of special methods. The outcome of these studies was to prove that there are separate biological types among hæmolytic streptococci, just as there are among other apparently closely related groups of microörganisms. More than 68 per cent. of the strains investigated comprised six easily distinguishable serological types.

This study was part of a general investigation of the biology of streptococcus, and, as a result of the facts developed, Bliss and Dochez[30] undertook a reinvestigation of the much debated ques-

tion of the unity of type of the S. hæmolyticus so constantly associated with scarlet fever. An effort was made to answer Jochmann's main objection to the etiological relationship of S. hæmolyticus to scarlet fever, namely, that the organism is not present in every instance of the disease, and that it cannot be satisfactorily differentiated from hæmolytic streptococci associated with the common septic conditions. Bliss [31] found when cultures are made from the throat early in the course of scarlet fever that hæmolytic streptococci are present in predominating numbers in 100 per cent. of individuals examined, thus confirming the earlier work of Baginsky and Sommerfeld. Immune sera were then prepared by the inoculation of rabbits with scarlet fever streptococci, and the capacity of these sera to agglutinate specifically a large number of freshly isolated scarlet fever strains was tested. Ten such sera were prepared from different strains of scarlet fever streptococci and each serum was found to agglutinate more than 80 per cent. of the strains isolated from scarlatinal throats. Agglutinating sera prepared from strains of hæmolytic streptococci derived from pathological sources other than scarlet fever in general, failed to agglutinate specifically the scarlatinal strains. Furthermore, strains of hæmolytic streptococci obtained from such conditions as tonsillitis, erysipelas, bronchopneumonia, and other septic diseases, as well as the various type streptococci, determined by Dochez, Avery and Lancefield were not agglutinated by the scarlatinal antistreptococcic sera. The evidence in favor of the specificity of the agglutination reaction of scarlatinal streptococci was reinforced by results obtained from agglutinin absorption experiments. Scarlatinal streptococcic sera also afforded some protection of experimental animals against virulent scarlet fever streptococci, but had no protective power against hæmolytic streptococci from other sources. This work indicates that the majority of hæmolytic streptococci found in association with scarlatina belong to a specific biological group and can, by appropriate methods, be distinguished from hæmolytic streptococci derived from other pathological conditions. These observations, I believe, confirm in a satisfactory manner the early studies of Moser and von Pir-

quet on the same subject. Contemporaneously with Bliss, Tunnicliff [32] investigated, by means of the opsonic and agglutination reaction, a series of hæmolytic streptococci isolated from patients during the early stages of scarlet fever. She concludes that the serum of sheep immunized with hæmolytic streptococci from the throat in the acute stage of scarlet fever contains opsonins and agglutinins for the hæmolytic streptococci that prevail in the throat and complicating lesions early in this disease, but not for hæmolytic streptococci from other sources, such as erysipelas, mastoiditis, measles, influenza, diphtheria and the normal throat. The results of her absorption experiments also indicate that the hæmolytic streptococcus from scarlet fever forms a distinct group, scarlatinal streptococci removing the opsonins and agglutinins for these cocci while absorption with a hæmolytic streptococcus from erysipelas has no such effect. These results also suggest that the hæmolytic streptococci from scarlet fever form a distinct serologic group. Somewhat later Gordon [33] found that eighteen strains of hæmolytic streptococcus isolated from scarlatina were identical in their agglutinative reactions. None of these strains absorbed the agglutinins from immune sera prepared from certain other types of hæmolytic streptococcus, designated by him as Types I and II. On the basis of this evidence, Gordon concludes that the streptococci from the throat secretions in scarlet fever constitute a group immunologically distinct from other varieties of streptococcus pyogenes. Eagles [34] in a recent study compared the serological reactions of hæmolytic streptococci from scarlet fever, puerperal sepsis, erysipelas and miscellaneous sources. He confirms the immunological specificity of the scarlatinal group and the clearness with which it can be separated from other types of streptococcus. He furthermore compared in an interesting manner a number of individual strains obtained at three to four day intervals from the same patient and demonstrated a gradual but progressive loss of specific agglutinability, a phenomenon which we have observed, and of which I shall say more later. Williams [35] has also studied the serological reactions of the scarlatinal streptococci and finds only 35 per cent. to belong to a single type, and is of the opinion that a greater

variability exists than is suggested by the work of the previous observers. Dick and Dick [36] have shown two strains of scarlet fever streptococci, one a mannite fermenter, and the other a non-mannite fermenter, to be serologically distinct, and believe that the agglutination reaction is of but little importance in determining the character of the scarlatinal streptococci.

It would seem, therefore, that the old question stressed by Jochmann, concerning the specificity of the streptococcus of scarlet fever still remains in dispute. The preponderance of evidence, however, strongly favors the belief that these cocci comprise a separate biological group and that the best method for determining this specificity of type is by agglutination. In order that satisfactory results may be obtained from this reaction certain rigid conditions must be complied with; spontaneous auto-agglutination must be prevented, and the streptococci in question must be studied fresh from their human environment. This latter requirement is of great significance. Recent studies by Avery and Heidelberger [37] have shown that the type specificity of pneumococcus is dependent upon the chemical constitution of the capsular substance. The production of this substance is a variable function of the organism; it is greatest in its strictly parasitic phase and is reduced by all factors which reduce virulence and lessen pathogenicity. That a similar loss of a specific function by scarlatinal streptococci takes place when they are removed from their parasitic environment is extremely likely. Bliss and Stevens and Dochez [38] have emphasized the rapidity with which specific agglutinating qualities are lost upon continued growth of these streptococci in artificial medium and Eagles suggests that the same change may take place under the influence of the immune bodies formed by a scarlatinal subject during convalescence. The suppression of specificity of serological reaction under the influence of immune bodies is, of course, a well recognized and established phenomenon among the pneumococci. The results obtained, therefore, indicate that if a large number of strains of scarlatinal streptococci are studied under appropriate conditions and within a short period of time from

their isolation during the acute stage of scarlet fever a high degree of serological specificity can be demonstrated.

Streptococcus scarlatinæ is found not only in the throats and organs of individuals suffering from anginal types of scarlet fever but has also been obtained from atypical forms of the disease, healthy carriers and contaminated food products. Serologically specific streptococci have been isolated from the local lesions in scarlet fever arising from the infection of wounds and burns, from the throat in scarlet fever without a rash, and from the lochial discharge in instances of puerperal scarlet fever. Bliss succeeded in tracing a small outbreak of scarlatina in an isolated children's institution to a recently admitted healthy carrier of Streptococcus scarlatinæ. Stevens and myself identified by means of agglutination and absorption reactions as scarlatinal streptococci organisms isolated both from the contaminated milk which had given rise to a milk-borne epidemic of scarlet fever, and from the throats of patients who contracted the disease from this milk. As a result of these studies the importance of Jochmann's objections to Streptococcus scarlatinæ as the etiological agent of scarlet fever was much lessened and students again began to take an active interest in this organism as the probable cause of the disease.

From the very beginning of the study of scarlet fever efforts have been made to produce the disease experimentally in animals and in man by inoculation with scarlatinal material. Most of these attempts have had in view the demonstration of an unknown virus of the filter passing type. Streptococcus scarlatinæ, in spite of the presumptive evidence in its favor and of the fact that some of the most typical examples of scarlet fever in animals have been associated with its presence has been but little tested for its capacity to produce the disease experimentally. Class [39] in 1899 reported the experimental production of this disease in swine by an organism designated by him as Micrococcus scarlatinæ. This was a gram negative coccus isolated from three hundred cases of scarlet fever and was, in all probability, a streptococcus. Krumwiede, Nicoll and Pratt [40] in 1914 observed an accidental infection of a laboratory worker, who sucked into her mouth a

mixture of living streptococci containing Streptococcus scarlatinæ. Three days later this individual developed a sore throat and subsequently experienced a typical attack of scarlet fever with all the usual phenomena. Because of the interest aroused by this observation, efforts were made to infect monkeys with the same streptococcus, but no instance of the disease was successfully produced.

In 1921 Dick and Dick [41] made a series of human inoculations with certain organisms obtained from the throats of individuals suffering from scarlet fever. Among the organisms utilized for this purpose was Streptococcus scarlatinæ. Though some of the volunteers experienced sore throats as a result of the treatment, no true instance of experimental scarlet fever resulted. In 1923 the same workers [42] repeated their efforts to produce scarlet fever in human volunteers. In the second series of observations a hæmolytic streptococcus obtained from the infected finger of a nurse suffering from wound scarlet fever was used for purposes of inoculation. Five volunteers were inoculated by swabbing the tonsils and pharynx with four-day-old cultures of the streptococcus in question. Three of these individuals remained without evidence of infection and one suffered from sore throat and fever without a rash. The fifth volunteer, however, who had been inoculated with the streptococcus after three weeks' growth in artificial medium experienced a typical but mild attack of scarlet fever, beginning forty-four hours after inoculation, and characterized by sore throat, general malaise, nausea, fever, leucocytosis, a typical rash and albuminuria. Desquamation began on the hands and feet on the tenth day, and was complete by the end of the fourth week. Five volunteers inoculated with filtrates of the above-mentioned organism remained well and showed neither sore throat nor rash. Subsequent inoculation of four of these volunteers with living unfiltered cultures of the original streptococcus resulted in the experimental production of another instance of scarlet fever. These observations were confirmed later by the same investigators [43] by the experimental production of another instance of scarlet fever in an individual proven susceptible by the use of a skin test devised by them.

In 1920 Dochez and Bliss, while studying the biological reactions of Streptococcus scarlatinæ, observed in a dog infected subcutaneously with living organisms, the development of an intense general erythema followed later by desquamation. Attempts to reproduce this phenomenon in dogs resulted in failure. Stevens and Dochez later tried other animals, including monkeys, without success. Failure in these instances seemed to be due to our inability to induce a local infection because of the low virulence of the organism for the animals employed. Finally Dochez and Sherman [44] were successful in producing in guinea-pigs and young swine a series of manifestations comprising some of the principal phenomena of scarlet fever. Successful local infection was achieved by injecting melted agar subcutaneously and infiltrating the mass with living culture of Streptococcus scarlatinæ. Since it had become increasingly evident that scarlatina has a certain resemblance to diphtheria, in that there is a local infection in the throat from which the specific toxic substance is distributed, we hoped that a similar absorption of toxic material would take place from the local area of infected agar. This proved to be the case and guinea-pigs and swine treated in the manner described developed an erythematous rash, fever, leucocytosis and progressive loss of weight. From eight to twelve days following infection the swine had general scaly desquamation and the guinea-pigs slight general desquamation and complete separation of the skin over the pads of the feet. This phenomenon could not be induced when hæmolytic streptococci from sources other than scarlet fever were utilized. Some of the guinea-pigs died acutely from the toxic substances absorbed from the locally infected area, and after death streptococci could not be demonstrated by culture either in the blood or serous cavities.

The production of experimental scarlet fever in human beings and in animals by inoculation with Streptococcus scarlatinæ had by this time made it increasingly likely that this organism is the causative agent of the disease. The evidence in favor of the absorption from the area of local infection of a toxic substance

which might be responsible for the clinical picture, had again brought into the foreground the analogy with diphtheria.

Investigators of scarlet fever have for many years been impressed with the similarity of this disease to diphtheria. Berge,[45] as early as 1895, suggested that scarlatina is due to a local infection in the throat with streptococcus and that the general symptoms of the disease are due, as in diphtheria, to the absorption into the general circulation of soluble toxins formed by the infecting microörganism at the site of the local disease. Gabritchewsky and his co-workers, in their studies of scarlatiniform manifestations which followed immunization of human beings against scarlet fever by means of vaccines of killed cultures of streptococcus scarlatinæ, attributed these reactions to the presence of a toxin in the vaccines. They drew attention to the absence of a vaccine erythema in individuals who gave a history of having had scarlet fever, and its failure to develop in patients during the period of convalescence from this disease.

Much evidence in favor of the existence of a soluble circulating poison in scarlet fever has also come from the study of the so-called Schultz-Charlton extinction phenomenon. In 1918 Schultz and Charlton [46] discovered that if one injects into the skin of a scarlet-fever patient with a bright red rash 1 cc. of serum from a normal person, or from a patient convalescent from scarlet fever, there appears after a time at the site of the injection a characteristic change. This change begins after about six hours and consists in a complete blanching of the rash over an area of from one-half inch to a few inches in diameter. In the affected area the swollen follicles, which are a feature of many rashes, disappear. Looked at from a distance, the margin of the defect in the rash is generally sharply defined. The color of the blanched area is that of normal skin and the duration of the typical phenomenon coincides on the whole with that of the rash itself. On the other hand, serum taken from scarlet-fever patients during the acute stage of the illness invariably gave negative results. Subsequent investigators abundantly corroborated the accuracy of the observation of Schultz and Charlton. As a result of these later studies it was established that the serum

of about sixty per cent. of normal adults possesses the capacity to blanch the rash in an active case of scarlet fever; that convalescent scarlatinal serum gives a positive rash extinction test in from 80 to 100 per cent. of instances; and that the serum during the active stages of scarlet fever never manifests blanching power. The Schultz-Charlton reaction was first used as a diagnostic test of scarlet fever, and the capacity to extinguish the rash in scarlet fever was believed to be due to a normal property of human serum, which is temporarily lost during the acute stage of scarlet fever and regained during convalescence.

In 1923 Mair [47] published a study of the Schultz-Charlton reaction in which he confirmed in general the observations of previous workers but gave the phenomenon a much more satisfactory explanation. He had an opportunity of studying the blanching power of the serum of a child both before and after an attack of scarlet fever and showed that the serum before the attack gave a negative Schultz-Charlton test, but during convalescence acquired the capacity to extinguish an active rash. This disproved the previous belief that a positive reaction was due to some property of normal human serum which is lost during the acute stages of scarlet fever. He also showed that the sera of young children who had not had scarlet fever give a negative reaction in a much greater proportion of instances than do adult sera and that the reactivity of the sera of new-born infants corresponds with that of the mothers.

Mair had been interested for some years in the resemblance of scarlet fever to diphtheria. As a result of his later work, he came to believe that the rash and other changes in the skin in scarlet fever are due to a scarlatinal toxin which has entered into combination with the tissue cells. Among the affected cells are those contractile elements which have been shown to exist even in capillary blood-vessels, and to the function of which the normal tone of the capillaries is due. The toxin interferes with the function of these cells and a loss of tone of the capillaries results in the erythema and exudative phenomena with which we are familiar in the scarlatinal rash. He supposes that the serum giving a positive Schultz-Charlton reaction contains an antitoxin

which is able to dislodge and neutralize the toxin fixed in the cells, and thus restores their normal function over the area injected. He adds that the true causal organism when discovered should be capable of producing a toxin, and that the immunization of animals to this poison should give rise to an antitoxin capable of producing a positive Schultz-Charlton reaction in man.

We also had been pondering over the analogy between scarlet fever and diphtheria and, at the time of the publicatoin of Mair's observations, had already produced in horses by immunization to Streptococcus scarlatinæ an antitoxic serum of the type postulated by him. Struck by the fact that occasionally in guinea-pigs inoculated for the production of experimental scarlet fever sufficient poison was absorbed from the local lesion to kill the animals acutely, we determined to make use of the method for the production of an antitoxic serum in horses. Masses of melted nutrient agar were injected beneath the skin and then infiltrated with increasing doses of Streptococcus scarlatinæ. The animals experienced a general reaction, and some of them, curiously enough, showed loss of hair and extensive general desquamation. After nine months' immunization the first animal was bled and his serum tested by Blake, Trask and Lynch [48] for its correspondence with human convalescent scarlatinal serum. When injected intracutaneously in a patient with a bright rash in the acute stage of scarlet fever this serum caused a complete extinction of the rash over an area five to ten centimetres in diameter. The blanching appeared in from six to twelve hours following injection of the serum and persisted throughout the course of the disease. As a rule, the characteristic pigmentation and desquamation were absent during convalescence over the blanched area. Antisera prepared from other hæmolytic streptococci and from Streptococcus scarlatinæ injected intravenously into animals, failed to induce blanching of the rash. Furthermore, scarlatiniform rashes in such conditions as erysipelas, measles, and other exanthematic diseases were not influenced by the intracutaneous injection of the scarlatinal antitoxin. Injection of a sufficient quantity of the serum intramuscularly in a patient in the exan-

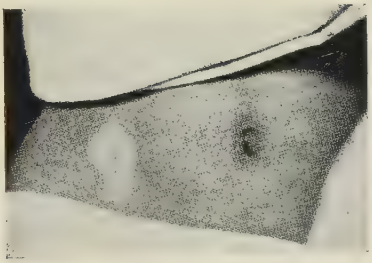

FIG. 1.—Blanching of skin in human scarlet fever due to intracutaneous injection of scarlatinal strep. antitox. (Blake, Trask & Lynch.)

FIG. 2.—Desquamation following experimental scarlet fever in guinea-pigs.

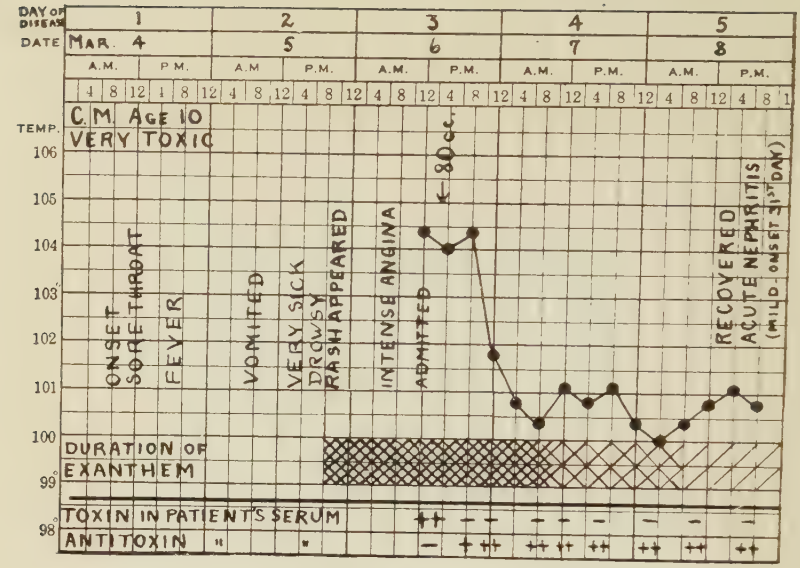


FIG. 3.—Analysis of effect of treatment of scarlet fever with scarlatinal streptococcus antitoxin. (Blake & Trask.)

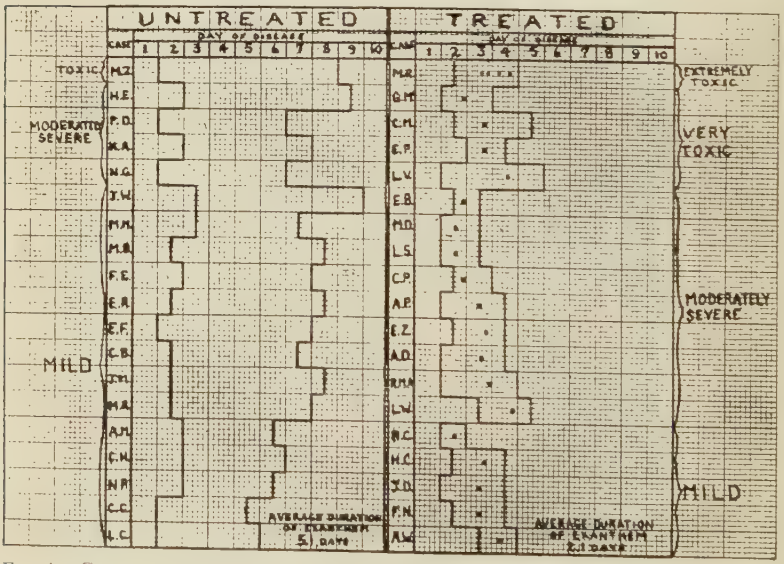


FIG. 4.—Comparative duration of rash in untreated patients and patients treated with scarlatinal streptococcus antitoxin. (Blake & Trask.)

thematous stage of scarlet fever causes a complete fading of the rash over the whole body in from twelve to twenty-four hours.

Blake and Trask [49] have demonstrated that there is present in the circulating blood and in the urine during the acute stage of scarlet fever a toxic substance which causes an erythematous reaction when injected intracutaneously in individuals whose blood serum gives a negative rash extinction test. This substance appears to be identical with the culture toxin of the Dicks and circulates in the blood for several days. When patients with scarlet fever having easily demonstrable amounts of this poison in the blood are injected with scarlatinal antitoxin, the circulating toxin is rapidly neutralized, a single dose of forty cubic centimetres causing its complete disappearance throughout the remaining course of the disease. The antitoxin quickly predominates in the blood and the treated patients' serum acquires the capacity to induce a positive Schultz-Charlton extinction test, a property that does not develop in untreated patients until late convalescence. The other toxic manifestations of the diseases are likewise favorably influenced. An immune horse serum, therefore, prepared in the manner described, seems to contain a potent antitoxin and behaves in every way in a manner similar to human convalescent scarlet fever serum.

The further studies of Dick and Dick [50] demonstrating the presence of a toxic substance in filtrates from blood broth cultures of Streptococcus scarlatinæ have brought to light a number of new and important facts which develop still further the analogy between scarlet fever and diphtheria. The toxic filtrate was obtained by these authors from a strain of streptococcus with which they had produced experimental scarlet fever in man. When individuals who give a negative history for scarlet fever are injected intracutaneously with small amounts of this toxin, within about six hours there appears at the site of inoculation a small circular area of erythema, which increases in size and intensity of color for from eighteen to thirty-six hours. Frequently the local reaction is accompanied by swelling of the skin. When a series of normal persons who have not had scarlet fever are injected with this substance, 41.6 per cent. of these

show a positive erythema reaction in the skin, a manifestation resembling the Schick test for susceptibility to diphtheria. The remainder who give a negative reaction are considered to be immune, because of the probable presence of circulating antitoxin in the blood, just as in the case of diphtheria. In addition, patients who are recovering from scarlet fever when tested intracutaneously with this substance, give but a very faintly positive or uniformly negative skin reaction. A similar condition of affairs is found to exist among those who have had scarlet fever at some earlier period of life. If individuals who have been proven susceptible to scarlet fever by means of the Dick test, are injected subcutaneously with larger amounts of the toxin, they exhibit certain of the toxic manifestations of the disease, such as nausea and vomiting, fever and an erythematous rash. When toxic filtrate is mixed *in vitro* with a small amount of convalescent scarlet fever serum, its capacity to produce a positive skin reaction is completely neutralized. Neutralization of the reaction was also obtained *in vivo* by the injection into susceptible human beings of larger quantities of convalescent serum. More recent studies of the Dicks [51] have shown that individuals who react positively in the skin can be immunized by repeated doses of the toxin, so that within a relatively short period of time the skin reaction becomes negative, and there is some evidence to support the belief that such individuals may be immune to the disease scarlet fever.

Zingher,[52] in an extensive study, has confirmed the observations of the Dicks and extended them somewhat. He has shown that the Dick reaction is positive in the early stages of scarlet fever in most instances, and that it becomes increasingly negative as the disease progresses through convalescence. He has, furthermore, drawn a very close analogy between the data obtained from the Schick test in diphtheria and those obtained from the Dick test in scarlet fever. In general, susceptibility to the latter reaction is greater in childhood and diminishes in adult life. There is also an inherited resistance to the toxin in infants whose mothers exhibit a negative reaction.

These studies, therefore, indicate that there is present in

sterile filtrates from cultures of Streptococcus scarlatinæ a toxic substance which bears a specific relationship to scarlet fever. By means of this substance it is possible to detect susceptibility in persons who have not suffered from scarlet fever, and furthermore to demonstrate the development of immunity in patients who are recovering from an attack of the disease. This work brings further strong support to the belief that Streptococcus scarlatinæ is the etiological agent of scarlet fever.

In 1921 Di Cristina,[53] in Italy, obtained from the blood of patients with scarlet fever an anærobic Gram-positive diplococcus. Other Italian investigators subsequently isolated a similar organism from the nasopharynx, bone marrow, spleen and desquamating skin of children with scarlet fever. This organism, on further study, was found to show specific serological reactions with the serum of recovered cases of scarlatina. Inoculation of children with living cultures of the organism is said to have produced an attenuated form of scarlet fever. Furthermore, prophylactic inoculation with killed cultures prevented the development of scarlet fever among a number of children exposed to the disease. Unfortunately, we are not in a position as yet to determine with any assurance the significance of this organism in scarlet fever, since an opportunity to study it bacteriologically has not been afforded.

Have we now reached the end of man's long struggle to find the cause of this interesting and at times formidable and dangerous disease? Personally, I think we have. Belief that scarlet fever may be caused by a protozoan parasite, or by one of the mysterious ultramicroscopic viruses, must, I think, be discarded in view of the fact that the evidence brought forward in support of the causative relationship of such types of microörganisms to the disease is entirely unconvincing. On the other hand, can we say with certainty that scarlet fever is caused by a type of Streptococcus hæmolyticus? Certainly a chain of evidence in favor of this organism has been patiently and progressively forged which is as strong as that in many diseases whose etiology is now accepted without discussion. The constant association of this organism with the primary and secondary manifestations

of the disease, its specific character, its capacity to produce the experimental disease in man and in animals, the ability of human convalescent scarlet-fever serum to neutralize the toxic effects of this streptococcus, the capacity of an antistreptococcus horse serum antitoxic in nature to counteract the specific toxic manifestations of the disease in man, and finally the isolation from Berkefeld filtrates of this streptococcus of a toxic substance which bears a specific relationship to immunity in scarlet fever, leaves little room to doubt that Streptococcus scarlatinæ is the principal and probably only etiological agent of scarlet fever.

Let us, therefore, be optimistic and assume that a just reward has come to those many soldiers in the army of science, too numerous to be mentioned in so short an exposition, and that another disease has been added to those about which the essential specific facts are known. Let us also hope that the methods of prevention and treatment based on these facts may prove as successful as the promising character of the preliminary work suggests.

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# THE INTESTINAL ABSORPTION OF ANTIGENIC PROTEIN *

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A FOREIGN protein absorbed without digestion entering the blood and body fluids is capable of causing toxic symptoms and elicits certain specific reactions. These reactions involve the production of antibodies related to the special protein and are an expression of its antigenic action.

My use of the term antigenic protein is to designate a native protein or its cleavage product which is capable of provoking the production of specific antibodies in the animal organism or of reacting in a specific way with such antibodies when once produced.

Investigations by Oppenheimer, Michaelis, Obermeyer and Pick, Wells, Gay and Robertson, Landsteiner, Fink and others have shown that only slight alteration of the protein molecule seems consistent with the preservation of antigenic properties. Although our knowledge of this subject is still incomplete, it seems probable that degradation products below the primary proteoses are apt to have little, if any, antigenic action.

The work of Fisher, Abderhalden, Folin and others has demonstrated that under normal conditions ingested protein is split largely into its constituent amino-acids before absorption occurs. Assuming that this applies to ingested protein in a quantitative way, the enteral absorption of protein with antigenic action must indicate deficient protein digestion or alteration of intestinal permeability.

Accurate observations on the absorption of antigenic protein have been possible only through the development of immunologic methods. Such methods are designed directly to demonstrate the absorbed protein in the blood or urine by means of specific pre-

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cipitating sera, or the power of the blood or urine containing the protein activity to sensitize guinea-pigs to subsequent injections of the same protein. Antigenic protein can be detected also by means of complement fixation tests.

Other methods depend on the presence in the blood of specific antibodies which may appear following the absorption of undigested protein. If antigenic protein entered the circulation it would probably cause the production of specific antibodies which, if present in sufficient concentration, could be detected by appropriate tests. Such tests are for precipitin, evidenced by the appearance of a precipitate when the blood serum is mixed in appropriate amount with a proper solution of the protein, or for the anaphylactic antibody by testing the capacity of the blood passively to sensitize a guinea-pig to a subsequent injection of the suspected protein. The complement fixation test may be used also to detect the presence of antibody. The value of such tests lies in their specificity. Antibody reacts only with the protein, causing its production, and antigenic protein reacts solely with its own specific antibody. There are certain limitations to these statements, but for the practical purposes with which we are concerned they are essentially correct.

Most of the literature on the absorption of antigenic protein from the gastro-enteric tract is descriptive of observations on the new-born or on infants suffering from nutritional disorders or diarrhœa. It is generally assumed that under normal conditions protein is completely digested in the intestine, and in consequence none is absorbed in antigenic form. To a large degree this assumption is supported by direct investigation, although a few observations in adults by Ascoli, Uhlenhuth, Michaelis and Oppenheimer and others showed evidence of absorption of undigested egg protein when very large amounts of egg white were ingested. Similar observations concerning the absorption of undigested egg protein by normal infants have been made by Lust, Hayashi, Schloss and Worthen, Lawatschek and others. Such observations, while differing somewhat in technique, were mostly negative, although in a small number of infants positive results were obtained by Lust, Hayashi and Schloss and Worthen.

Tests of this kind, which involve feeding large amounts of egg protein, usually in a single dose, are hardly comparable to the administration of protein in ordinary food mixtures. Therefore, investigations designed to determine the presence of antigenic milk protein in the blood or urine of normal infants fed on cow's milk have been carried out by Hamburger, Kleinschmidt, Moro, Bauer, Krasnagaski, Schloss and Worthen, Modigliano and Benini and others. The results were uniformly negative, and show that under ordinary circumstances antigenic milk protein is not absorbed by normal infants in sufficient amount to be detected directly by precipitin or anaphylactic tests.

### I. THE INTESTINAL ABSORPTION OF ANTIGENIC PROTEIN BY THE NEW-BORN

There is a general belief that the intestinal tract of the new-born is readily permeable to undigested protein. A review of the available data gives rise to much doubt as to the complete accuracy of this conception, and while it would seem hardly profitable to enter into a detailed discussion of the literature, much of which is polemical, it may be of sufficient interest to review briefly the salient points.

A considerable number of the observations concerned the absorption of antitoxin when given by mouth. It was assumed that the antitoxic antibody was either a true protein or inseparably united to protein, and in consequence its appearance in the blood after ingestion was considered proof of absorption of undigested protein.

Investigations of this type were conducted on new-born animals by Römer, v. Behring, Römer and Much, Uffenheimer and others, and demonstrated that antitoxic antibody was absorbed from the gastro-enteric tract when it was contained in the milk of the lactating mother but not when contained in the serum of another animal. Experiments by Salge on young infants admit of similar conclusions. Diphtheria antitoxin contained in the milk of the immunized mother or wet nurse was absorbed, but no absorption occurred after the ingestion of antitoxic horse serum or goat's milk containing typhoid agglutinin or diphtheria

antitoxin. Observations by other workers were in agreement with those cited.

Assuming that the absorption of antitoxin indicates the absorption of protein these experiments demonstrate the permeability of the intestine of the new-born to homologous protein and do not warrant the general conclusion that the intestine of the new-born is regularly permeable to foreign protein.

Direct tests of the permeability of the intestines to foreign protein have been made on new-born laboratory animals and on new-born infants by Uffenheimer, Ganghofner and Langer, Hamburger and Speck, Lawatschek, Modigliano and Benini and others. The investigations involved the attempt to demonstrate egg, serum, or milk protein by precipitin tests in the blood or urine, and led to irregular results and controversial literature difficult to appraise. The absorption of undigested milk protein by the new-born was not demonstrated by any observer, but several workers, notably Lawatschek, Hayashi and Ganghofner and Langer, demonstrated the absorption of antigenic egg protein. Essentially negative results with egg protein, however, were obtained by Hamburger and Speck and by Uffenheimer. These discordant results only justify the conclusion that after the ingestion of relatively large amounts of egg white by the new-born, egg protein may be demonstrated in the blood or urine, but they do not prove the general principle that the intestine of the new-born is regularly permeable to heterologous protein ingested in physiological amounts.

### II. THE INTESTINAL ABSORPTION OF ANTIGENIC PROTEIN BY INFANTS WITH DIARRHŒA

In contrast to the equivocal and uncertain results on the enteral absorption of antigenic protein by the new-born, the investigations on infants suffering from diarrhœa, especially in its severer forms, leave little doubt that in this condition antigenic protein is frequently absorbed from the gastro-enteric tract.

In the course of investigations on animals a number of observers have found that chemical irritation of the intestines provoked the absorption of antigenic protein demonstrable in the

blood or urine by means of precipitin tests. It seems hardly profitable to consider this data in detail, since there are a sufficient number of observations directly concerning infants with diarrhœa.

A number of workers have conducted tests to determine the possibility of the enteral absorption of undigested egg protein by infants suffering from ordinary fermentative diarrhœa. Egg white was fed and the blood or urine treated with an anti-egg rabbit serum to detect the presence of precipitable egg protein. Such investigations conducted by Lust, Hayashi, Schloss and Worthen, Lawatschek and others gave positive results in a large percentage of the cases. The presence and degree of absorption were proportional to the severity of the diarrhœa and prove definitely the enteral absorption of antigenic egg protein in this condition.

These observations in most instances followed the ingestion of a single relatively large dose of egg-white, and do not prove that the same condition obtains for milk protein given regularly in the food. The investigations on the permeability of the intestine to milk protein are therefore of more practical significance. One of the great difficulties in examining the body fluids for milk protein by the precipitin reaction is due to the relatively weak antigenic action of milk protein. When rabbits are immunized to milk in the usual manner their serum rarely has the capacity of precipitation when mixed with milk diluted more than 800 to 1000 times, while under similar conditions egg-white provokes the production of sera capable of showing a precipitate with egg-white diluted 100,000 to 500,000 times.

Tests with the usual anti-milk sera applied to the blood or urine of infants with diarrhœa by Kasnogoski, Kleinschmidt, Hamburger, Moro, Bauer, Schloss and Worthen and others gave entirely negative results. The only positive results reported from the use of sera of this type were those of Modigliano and Benini. They used an anti-casein serum with a titre of 1–1000, and by its use were able to detect casein in the blood of a few

infants with severe diarrhœa. It is difficult to interpret their statistics accurately, owing to the terminology used. While the results of Worthen and myself were entirely negative with the usual weak anti-milk sera, we were able to obtain one lot of serum with a titre of 1–20,000, which gave positive results in two of the four cases of diarrhœa investigated.

In 1923 Anderson and I reported the results of investigations bearing on the occurrence and frequency of the enteral absorption of antigenic milk protein. Since this and more recent investigations by Anderson, Miss Myers and myself will be referred to frequently from now on, it will avoid repetition to cite an important technical detail. One of the great difficulties encountered in tests for milk precipitin by previous observers and in our own earlier work was to obtain a milk solution sufficiently clear to reveal the presence of slight or moderate precipitates. Ordinary milk gives very turbid solutions even in high dilution and only marked precipitates can be detected. We found that a commercial brand of dried milk gave very clear solutions in which slight precipitates could be detected readily and permitted the use of tests for precipitin with assurance and accuracy.

Thirty-three cases of moderate or severe diarrhœa were observed by Anderson and myself, and milk antigen was demonstrated in the blood of eighteen by either the precipitin or anaphylactic test. Despite the fact that antigen was found in the blood of only approximately one-half of the cases, tests for antibody showed that absorption of antigenic protein occurred in a much larger percentage than its direct demonstration indicated. This was shown by the presence of precipitin for milk protein in the blood of most cases after the subsidence of the diarrhœa. For example, in seventeen cases of diarrhœa the blood contained no precipitin for milk during the disease but precipitin was found in the blood of all, 10–14 days later. The direct test for antigen was positive in only eight of these seventeen cases. The difference in the results of the tests for antigen and precipitin is probably due to several factors. Antigen is probably absorbed irregularly and may not be present in the blood at all times in

sufficient amount to give a positive reaction. Also, it is possible that antigen is quickly anchored by tissue cells, or antibody, and thus removed largely from the circulating blood in the same manner as it is supposed to be removed in the experimental animals. Moreover, owing to the relatively weak precipitating action of anti-milk sera small amounts of antigen may be missed. On the other hand, precipitin once formed is present in the blood for some time and hence can be demonstrated readily (Fig. 1).

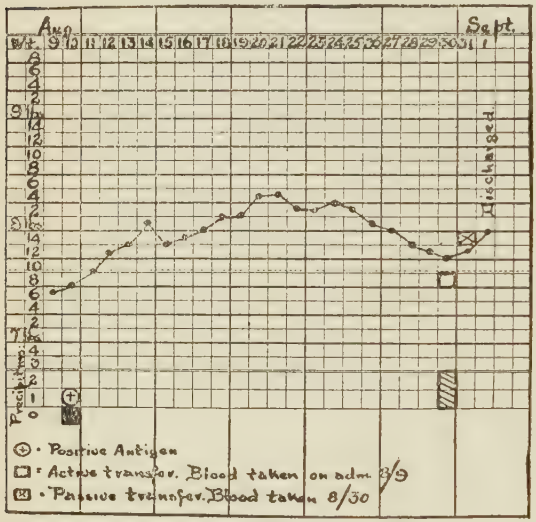


FIG. 1.—(*From the American Journal of Diseases of Children*, 1923, xxvi, 451). This figure illustrates the presence of antigenic cow's milk protein in the blood during acute diarrhœa and the subsequent development of precipitin and anaphylactic antibody for cow's milk protein.

On August 8th the precipitin test for cow's milk was positive. In confirmation of the precipitin test, the patient's blood actively sensitized a guinea-pig to cow's milk. The animal developed fatal anaphylactic shock following an intravenous injection of milk 21 days after a preliminary injection of the patient's blood.

Although the blood contained no precipitin for cow's milk on August 8th, precipitin was present on August 30th probably due to the previous absorption of antigenic cow's milk protein. At this time anaphylactic antibody was demonstrated by the passive sensitization of a guinea-pig by the patient's blood.

This demonstration of the frequency of absorption of antigenic milk protein in diarrhœa leads naturally to a consideration of its clinical importance. Upon purely hypothetical grounds the absorption of antigenic protein may be of influence either directly, by causing toxic symptoms, or remotely by inducing sensitization, thereby laying the groundwork for allergic disturbances should subsequent absorption of antigenic milk protein occur. Any discussion of the latter possibility at this time must be largely discursive, owing to lack of

adequate information, and therefore can be of little profit. Certain phases of this question which are of general interest will be discussed a little later.

The idea that the direct toxic action of enterally absorbed antigenic protein may play a rôle in causing symptoms during diarrhœa has been the subject of some discussion. Schlossmann, in 1906, on entirely hypothetical grounds, sponsored the view that absorption of milk protein from the intestinal canal was responsible for nutritional disturbances in infants. Kleinschmidt and others have advocated the hypothesis that such protein absorption may cause the fever and toxic disturbances often associated with severe diarrhœa. In discussion of this subject one is confronted with a number of complex problems for consideration. During severe diarrhœa there is a marked loss of water from the organism which must cause pronounced alteration of many important physiological processes. Resulting from this water loss are disturbances of the acid base balance capable also of causing severe symptoms. Marriott considers that all of the symptoms which may accompany diarrhœa can be explained by the dehydration. Bessau also believes that the dehydration is of considerable importance both directly and indirectly by increasing the permeability of the intestine to toxic bacterial products. Moro has assumed that the intestinal changes in diarrhœa permit the absorption of proteose or peptids which cause toxic disturbances.

The available evidence does not furnish any direct proof that the absorption of antigenic protein is responsible for the toxic symptoms which may accompany infantile diarrhœa. It is known, however, that parenteral injections of small amounts of protein can cause severe toxic symptoms, and there is reason to believe that undigested protein absorbed from the intestines can act in the same fashion. On the basis of this evidence, it would not seem to entail too great a stretch of imagination to conceive the possibility that absorption of undigested protein may be a prominent factor in causing toxic symptoms during infantile diarrhœa. An exact appraisal of its importance, however, is impossible at the present time.

### III. THE INTESTINAL ABSORPTION OF ANTIGENIC PROTEIN BY INFANTS WITH MARASMUS

Another and perhaps the most important condition in which antigenic protein is absorbed from the gastro-enteric tract is that termed variously marasmus, athrepsia or atrophy. With the advent of knowledge concerning the immunological reactions to foreign protein, the toxic action of such protein and its rôle in causing anaphylactic phenomena the suggestion arose that the deleterious effect of cow's milk on young infants was perhaps related to the absorption of undigested protein. This view was sponsored by Schlossmann on purely hypothetical grounds and in the absence of direct evidence acquired by himself or others to prove that such absorption did occur. Hamburger, Salge and others, partly on hypothesis but also on the basis of negative results arising from experimental work, considered that the absorption of significant amounts of milk protein in marasmus was very improbable. Hamburger's chief argument was that absorption of more than the merest trace of milk protein should provoke the occurrence of precipitin in the blood, which could not be demonstrated. More recently, however, definite evidence of absorption of antigenic milk protein by marasmic infants has been obtained.

*a. The detection of antigenic protein in the blood of infants with marasmus.*

Experiments to determine the permeability of the intestine of atrophic infants to egg protein have been made by Lust, Hayashi, Schloss and Worthen, Lawatschek and others. These investigations all showed that in a relatively large percentage of infants affected by severe marasmus the ingestion of moderate or large amounts of egg-white led to the presence of precipitable egg protein in the blood or urine. These experiments have an important bearing on the general aspect of the subject but do not prove that the absorption of milk protein would occur under the same conditions. Investigations concerning the absorption of milk protein have been along two main lines, one represented by the attempt directly to demonstrate the protein itself in the

blood or urine by precipitin or anaphylactic tests, the other to demonstrate specific precipitin or anaphylactic antibody in the blood. Direct tests for antigenic milk portein in the blood or urine of atrophic infants fed cow's milk have been largely negative. With the usual relatively weak anti-milk rabbit sera the observations of Moro and Hamburger, Bauer, Krasnogoski, Schloss and Worthen, and Anderson and Schloss, did not show the presence of antigenic milk protein in the blood or urine. The only positive results obtained by sera of weak titre were those of Modigliano and Benini who demonstrated casein in the blood of a small number of marasmic infants. The positive results of these workers compared with the negative tests of others cannot be explained. By means of the stronger serum referred to previously, Schloss and Worthen obtained positive tests for milk antigen in two of the four investigations on atrophic infants.

*b. The presence of precipitin in the blood of infants with marasmus.*

The results of tests for precipitin are of more interest. In 1902 Moro and Hamburger conducted tests for precipitin for cow's milk on atrophic infants with only negative results. Four years later Moro reported further observations conducted on 22 cases with two positive results. Bauer in the same year reported the demonstration of precipitin in the blood of an atrophic infant. The report of Bauer's case, however, stamps it as one of recurrent diarrhœa rather than of true atrophy.

In 1923 Anderson and I reported the results of tests for milk precipitin facilitated by the use of solutions of dried milk. We found that precipitin for cow's milk was present at some time in the blood of nearly all atrophic infants. To be exact, examination of the blood of 98 cases gave positive results for precipitin in 80. The tests on individuals were frequently negative, but when the patient was observed for some time positive results were practically always obtained. Subsequent investigations during the past 18 months by Anderson, Miss Myers and myself have confirmed the earlier results. This presence of precipitin in practically every case of marasmus must signify that antigenic

milk protein is regularly absorbed. The possible explanations for the failure directly to detect the absorbed protein were considered in the discussion of diarrhœa.

*c. The presence of anaphylactic antibody in the blood of infants with marasmus.*

In the experimental animals it has been generally observed that anaphylactic antibody capable of sensitizing passively another animal usually accompanies precipitin in the blood. Indeed, there is considerable evidence to show that the two antibodies are the same. It is beyond the bounds of my subject to enter this discussion, but since precipitating sera are usually able to confer sensitization it seemed of sufficient importance to conduct such tests. The blood of 30 marasmic infants containing precipitin to cow's milk was used to determine whether it would passively sensitize guinea-pigs to cow's milk. The result was positive in 14 and suggestive in 4.

Probably the most definite and clear-cut evidence of anaphylaxis is furnished by the Schultz-Dale phenomenon and for this reason it seemed of sufficient interest to apply this method.

The technic was essentially that of Dale. Virgin guinea-pigs were given intraperitoneal injections of precipitin-containing blood serum from marasmic infants. Within 24 hours the animals were killed quickly and one of the uterine horns was suspended in oxygenated Locke's solution and with one end attached to a lever writing on a slowly revolving smoked drum. The antigen was added directly to the bath of Locke's solution. Tested by this method, positive results were obtained in 5 cases.* An illustrative tracing is shown in figure 2.

The data given serve to establish the fact that the blood of marasmic infants may contain anaphylactic antibody. They

* Positive results were obtained in only a comparatively small number of the total tests. Sera which passively sensitized guinea-pigs so that a subsequent intravenous injection of antigen caused marked symptoms, frequently failed to elicit a positive Dale test. This is due in part to the difficulties inherent in the method. The uterine strip method was never positive unless the serum with which the animal was injected had a very high precipitin titre.

show further that the antibody from man is capable of affecting guinea-pigs in the same manner as the antibody from one guinea-pig affects another, thus offering indisputable confirmation of the previous observations of Bruck, Schloss, Schlutz and Larson, Longcope and Rackemann and others.

*d. The occurrence of cutaneous hypersensitiveness in marasmic infants.*

In view of the fact that in many instances the parenteral absorption of foreign protein leads to skin hypersensitiveness both in animals and in man, it seemed of interest to determine whether the enteral absorption of antigenic protein has the same effect. In connection with earlier work, scratch tests with cow's milk protein were made on a large number of marasmic infants. These tests were uniformly negative. It was realized then that the reaction to a scratch test would probably not be as marked as to an intradermal injection, but it was considered that the difference in sensitivity was probably merely one of degree and not sufficient to interfere with the practical value of the scratch test. The incorrectness of this view has been established by recent investigations of Robert DuBois, who, working in collaboration with Anderson, Miss Myers and myself, has found that many infants who develop precipitin to foreign protein give a definite skin reaction by the intradermal method and none to a scratch test. These observations are in accord with those of Cooke and his co-workers, Brown and others, and opposed to the view held previously by me.

FIG. 2.—(*From the Journal of Diseases of Children*, 1923, xxvi, 451). (Case B.C) Positive Dale test. A guinea-pig was passively sensitized by an injection of 2.5 cc. of serum (inactivated by incubation at 56C for one hour): 0.25 cc. of milk (0.012 gm. of casein) added at point designated C.M. produced complete contraction. Precipitins were 4 plus. The time is indicated in minutes.

The frequency with which an intracutaneous reaction to milk occurs is shown by the following statistics. Intradermal tests to milk protein were applied by DuBois to 26 marasmic infants, all of whom developed precipitin to cow's milk. The cutaneous

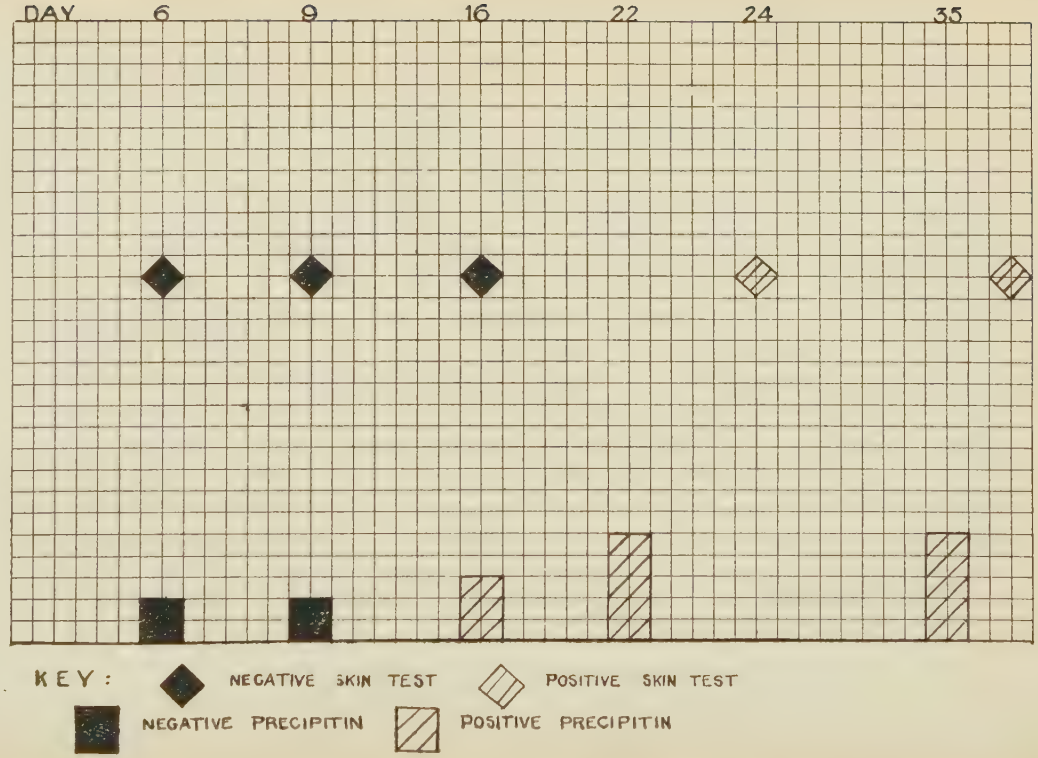


FIG. 3.—This graph illustrates the development of cutaneous hypersensitiveness to cow's milk and the development of precipitin for cow's milk in the blood of a marasmic infant.

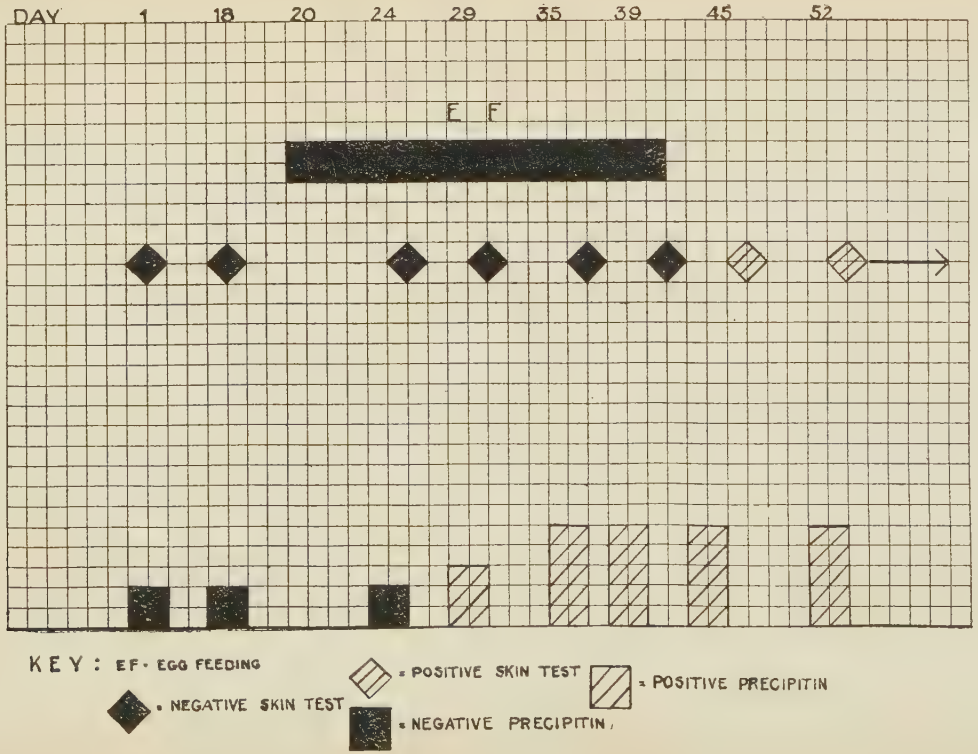


FIG. 4.—This graph illustrates the development of cutaneous hypersensitiveness to egg white and the development of precipitin for egg white in the blood of a marasmic infant following feeding of egg white.

reaction was positive in 12 of these 26 cases. The possibility of congenital skin hypersensitiveness was excluded in these cases for all of the infants were tested repeatedly and the positive skin test developed while they were under observation. The development of skin hypersensitiveness to milk is apparently less frequent in atrophic infants than the appearance of precipitin for milk protein in the blood. For example, a positive cutaneous reaction appeared in slightly less than half of the patients who developed precipitin (Fig. 3). More exact information concerning the comparative frequency of precipitin formation and cutaneous reaction were derived from experiments in which egg was fed. In those investigations exact amounts of egg protein were ingested for a definite period and the patient subjected to tests for cutaneous hypersensitiveness and for precipitin in the blood. Such observations were made on 20 marasmic infants and in 14 cutaneous hypersensitiveness developed. All showed the development of precipitin (Fig. 4).

The investigations on the development of cutaneous hypersensitiveness are too recent to determine definitely for how long a period the skin remains sensitive. In many cases cutaneous hypersensitiveness disappears within two months. In others, however, it persists for a much longer time. This phase of the subject will be considered fully in the complete report of the investigation.

Most important, however, for our present purpose is the fact that the enteral absorption of antigenic protein leads, not only to the appearance of specific precipitin in the blood, but also to specific cutaneous hypersensitiveness. On analysis of the two reactions it became evident that a cutaneous reaction rarely developed in a case in which precipitin was not present or did not ultimately appear. The time of appearance of the two reactions varied, but in some cases they were evident almost simultaneously. In the experiments with egg particularly, the devlopment of a cutaneous reaction bore relation to the degree of precipitin formation. These circumstances seem analogous to the animal experiments of Opie, from which he obtained evidence that the Arthus phenomenon is due to the interaction of anti-

gen and precipitin at the site of injection and was probably a protective mechanism which prevented the absorption of antigenic protein. Opie showed that the local reaction at the site of injection prevented antigen from reaching the blood-stream, which occurred regularly in animals not previously treated with antigenic protein.

*e. Discussion.*

In the discussion of precipitin formation it was pointed out that precipitin to cow's milk was not present in the blood of marasmic infants at all times, but that in many cases positive results were obtained only after repeated examinations. In the publications previously cited, Anderson and I called attention to a definite correlation between the detection of precipitin for milk protein in the blood of marasmic infants and the weight curve. During the period of stationary weight, as a general rule, precipitin could not be detected in the blood. Usually the tests for precipitin were positive almost simultaneously with increase in weight and with continuation of the gain in weight the precipitin disappeared. This correlation has been confirmed in subsequent studies by Anderson, Miss Myers and myself, and while the association cited is not invariable, yet, when the influence of intercurrent infections is taken into account, the relationship between precipitin and weight is sufficiently constant to relieve all suspicion that it may be accidental (Figs. 5 and 6).

In explanation of these observations, Anderson and I formulated the following hypothesis: During the period of stationary weight the intestine is permeable to foreign protein, which may act in a deleterious manner. After a varying period the absorbed protein provokes the production of precipitin which either alone or in combination with other agencies operative at the same time serves to mitigate the toxic influence of the absorbed protein. A gain in weight then ensues. With improvement, foreign protein is no longer absorbed, and the precipitin disappears. This conception that precipitin may exert a protective action is entirely in accord with observations on experimental animals. Weil, Dale, Opie and others have brought forth evidence to show that the development of precipitin in animals treated with for-

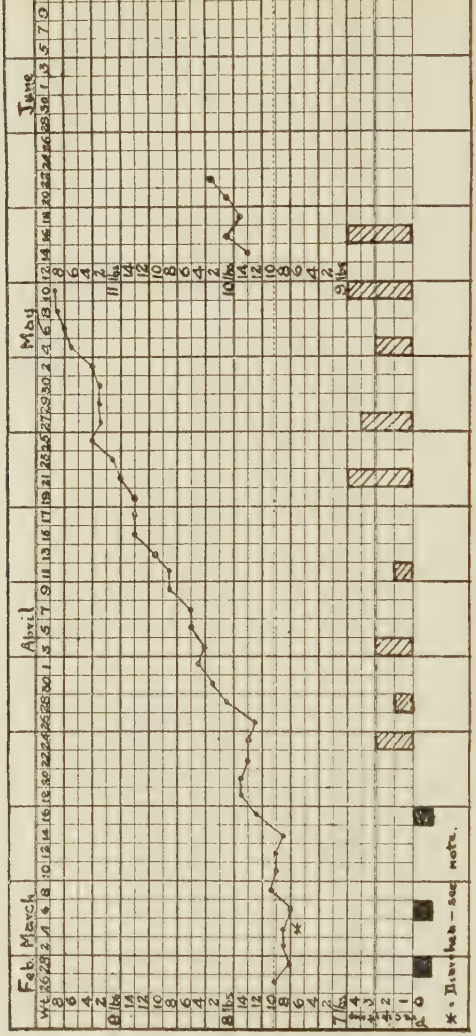


FIG. 5.—(*From the American Journal of Diseases of Children*, 1923, xxvi, 451). Precipitins were absent during the period of stationary weight and appeared when the child was gaining weight.

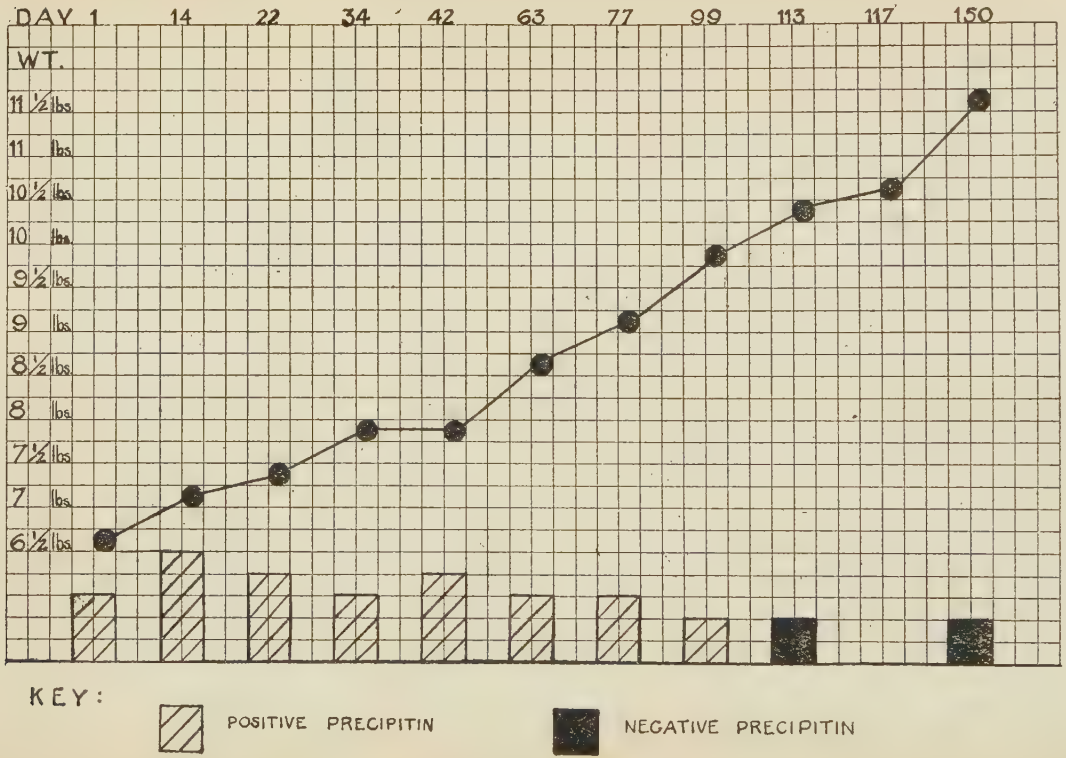


FIG. 6.—Illustrating the presence of precipitin for cow's milk in the blood of a marasmic infant during the stage of reparation and the disappearance of precipitin with continued gain in weight.

eign protein is probably expressive of a protective mechanism which serves to ward off toxic effects of subsequent absorption of antigenic protein. Longcope and Rackemann, in observations on serum sickness, found evidence that precipitin may play a part in bringing about recovery.

At the time we advanced the explanation cited it was realized that there were possible explanations for the irregularity in the detection of precipitin other than the one considered. For example, the failure to demonstrate precipitin in the blood of marasmic infants during the pre-precipitin period may not be due to the absence of precipitin from the blood but merely to the circumstance that antigen enters the blood at this time with sufficient rapidity to combine with the precipitin, thus preventing its detection.

Similarly, the detection of precipitin during the period of weight increase may not indicate its appearance at that time but merely that antigenic protein is no longer absorbed. In this event the precipitin which had escaped detection due to its combination with protein would now be free and detected readily by the precipitin test.

It seemed of sufficient importance to attempt to ascertain which of these views is correct, and experiments were carried out by Anderson, Miss Myers and myself with this object in view. If the failure to detect precipitin during the pre-precipitin period is due merely to the circumstance that it is masked by combination with antigen it seemed probable that if milk antigen were eliminated from the intestine the precipitin would be free and could be detected in the usual manner. Accordingly, five infants in the pre-precipitin stage were fed on mixtures free from milk protein for several days. In four of the five infants a diet free of milk protein did not lead to the appearance of precipitin in the blood. In one of the infants a trace of precipitin appeared at the end of 15 days but was evident on only a single test. After resumption of milk feeding for some time all of the infants showed definite precipitin formation. (Fig. 7.) These observations indicate that the failure to detect precipitin for milk

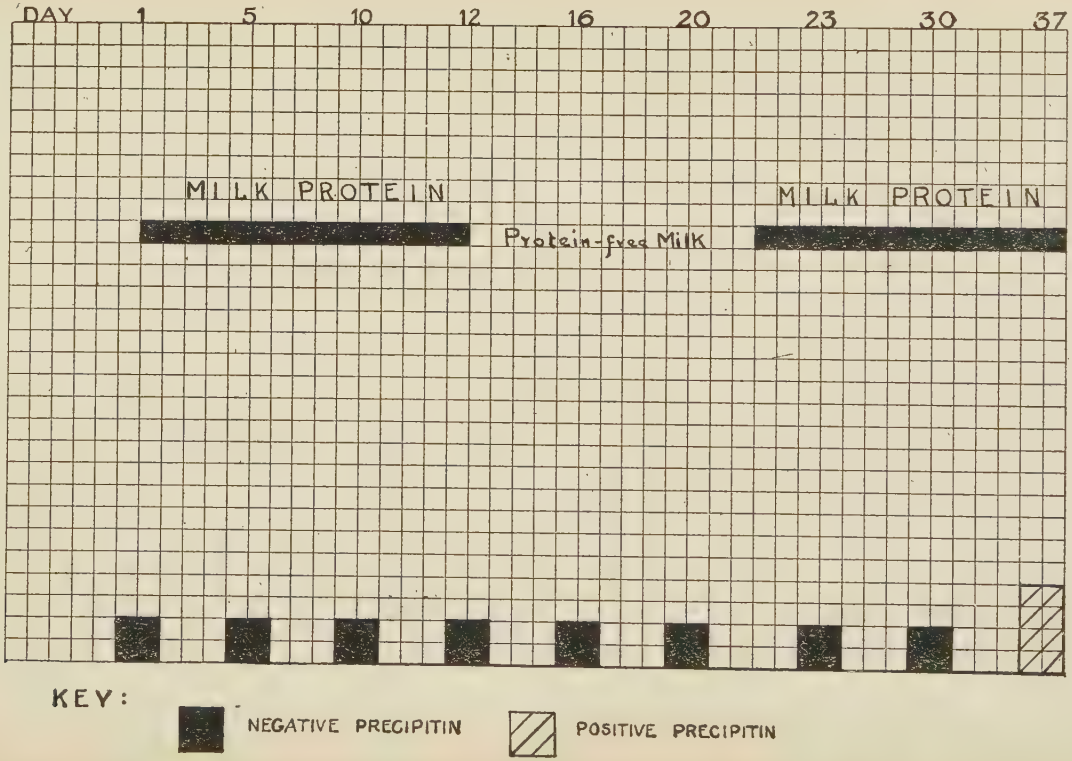


FIG. 7.—Illustrating that the absence of precipitin from the blood during the active stage of marasmus is not due to the masking effect of antigen. The elimination of protein from the diet of this infant did not result in a positive precipitin test.

during the active stage of marasmus is due probably to its absence and not to a masking effect of antigen.

Other experiments were designed to determine whether the period during which precipitin is detected in the blood of atrophic infants marks the first production of precipitin or whether it represents only the time at which the intestine becomes impermeable to milk protein, thus rendering precipitin free instead of combined with antigen.

The permeability of the intestine for antigenic protein during the period when precipitin to milk was present was tested by feeding egg-white and sheep serum for several days. It was found that a considerable number of the infants observed developed precipitin for egg or sheep protein 10 to 14 days after these proteins were first fed, thus indicating the absorption of antigenic protein. These observations demonstrate that the intestinal tract of marasmic infants is permeable to the proteins of egg-white and sheep serum at the time when precipitin to cow's milk can be demonstrated. This would seem to indicate that the appearance of precipitin to cow's milk is not due merely to the loss of intestinal permeability to antigenic cow's milk protein. Such evidence, however, is not conclusive if it is possible for the intestinal tract to be permeable to the proteins of egg-white and sheep serum after losing its permeability to the protein's of cow's milk. Indeed, evidence to be cited later indicates the possibility of such specific permeability. Despite the difficulty of arriving at a definite conclusion at the present time, it seems probable that the appearance of precipitin for cow's milk in the blood of marasmic infants is not merely incidental to the fact that antigenic protein is no longer absorbed but represents approximately the time when precipitin is first formed.

Although considerable direct evidence is still lacking, it seems justifiable for the present, at least, to retain our original conception, that with reference to the presence of precipitin in the blood there are three stages in infantile atrophy. During the first or active stage, characterized by stationary or declining weight, there is no precipitin in the blood and presumably antigen is being absorbed from the intestine. The second stage is

one of reparation with gain in weight marked by the appearance of precipitin for cow's milk in the blood. The third stage is characterized by disappearance of precipitin, due probably to the fact that its presence is no longer excited by absorption of antigenic protein. In accordance with accepted belief it seems probable that the production of precipitin represents a defensive mechanism.

It seems difficult to conclude the discussion of marasmus without reconsidering briefly the possible relation of the absorption of heterologous protein to the clinical manifestations of the disease. Conclusive evidence that the nutritional disturbance which is the predominant feature of marasmus is due to the injurious effect of the absorbed milk protein is lacking. All evidence is indirect and can be summarized briefly. It is known that parenteral injection of heterologous protein can exert a harmful influence. It has been shown that heterologous protein is absorbed by atrophic infants during the active stage of the disease. The development of precipitin marks the period of improvement, despite the fact that there has been no change in the quality or quantity of the food ingested. That this absorption of foreign protein may and probably does exert a deleterious effect is in accord with all available information, but how large a part it plays must be determined by further investigation.

### IV. INTESTINAL ABSORPTION OF ANTIGENIC PROTEIN BY NORMAL INFANTS

A number of circumstances have indicated the desirability of reinvestigating the possible absorption of antigenic protein by normal infants. It will be recalled that the conception of the impermeability of the normal intestine to foreign protein was based largely on negative tests for antigen in the blood or urine of individuals who had been fed on cow's milk for some time or after giving a single test feeding of egg protein. It is true that normal infants fed on cow's milk or egg for a long period have no precipitin for these proteins in their blood, but such observations do not exclude the possibility that precipitin had been

present at some time and later disappeared. Owing to these considerations, observations were conducted by Anderson, Miss Myers and myself on normal infants and children by feeding to them moderate amounts of a protein which they had never ingested before and carefully examining the blood for the appearance of specific precipitin. The first observations were conducted with egg-white. Thirteen normal infants, one to seven months old, who had never ingested egg, were fed 6 to 12 grams of powdered egg-white mixed with the day's feedings for periods which varied in different cases from 5 to 21 days. Precipitin for egg protein appeared in the blood of all of these infants. In 10 of the 13 precipitin was detected from 9 to 14 days after the egg was added to the diet. In two precipitin was not detected until one month, and in one not until six weeks after the commencement of the egg feedings.

Tests for cutaneous hypersensitiveness were made by DuBois on the same infants and six showed a positive reaction which appeared from 11 to 30 days after the egg feeding was begun (Fig. 8).

It is generally considered, and not without some foundation, that egg-white is apt to have an irritating effect on the intestinal mucosa which may predispose to absorption of the protein in antigenic form. For this reason similar tests were made with sheep serum. The general procedure was exactly the same as with egg, from 3 to 14 days, 50 cc. of sheep serum were mixed with the day's feedings. Due to technical and other difficulties it was possible to conduct satisfactory tests on only three infants. They were between 6 and 12 months of age. Precipitin to sheep serum was demonstrated in their blood 7, 13 and 19 days, respectively, after the feeding was commenced. None of these infants developed a cutaneous reaction to sheep serum during the period of observation.

The investigations with egg-white and sheep serum were conducted entirely on infants under one year of age, owing to the fact that beyond this time these substances may enter into the diet. In order to conduct experiments on older individuals it was necessary to use a protein-rich food which had not been

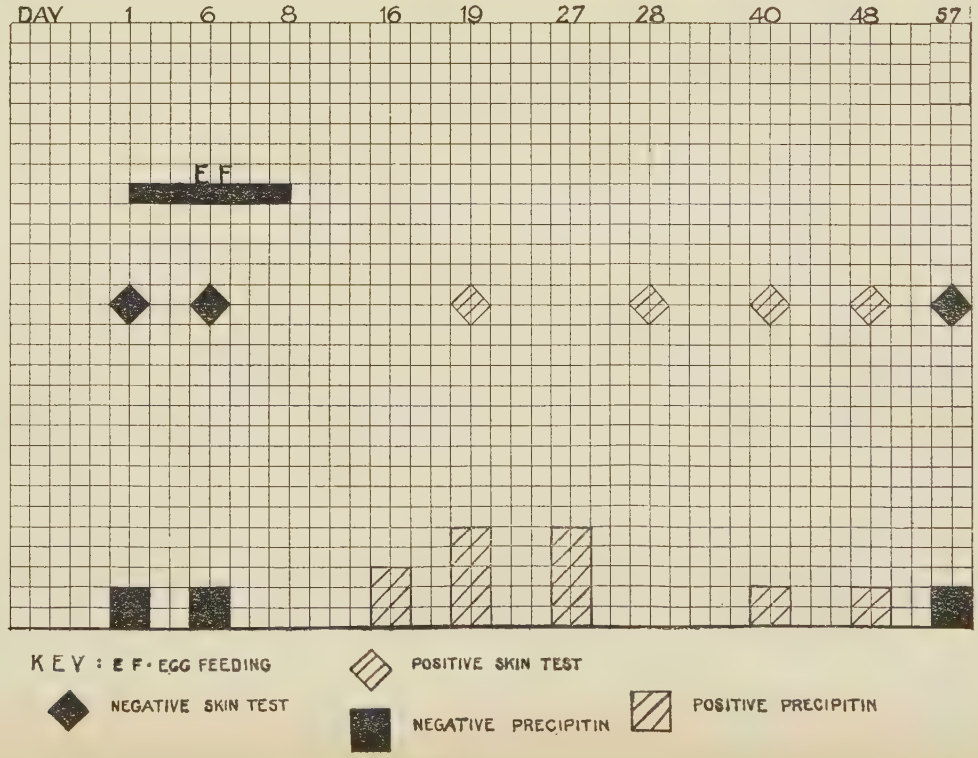


FIG. 8.—Illustrating the development of cutaneous hypersensitiveness to egg white and the appearance of precipitin for egg white in the blood following the first ingestion of egg white by a normal infant.

ingested previously. For this purpose almond was selected, owing to its richness in protein with strong antigenic action and also for the reason that its main protein—the globulin—is sufficiently soluble to use for precipitin tests.

Observations were made on 12 infants, to whom 15 to 25 grams of powdered almond were fed in 24 hours. Seven of them were over one year of age and five were under one year of age. Precipitin for almond protein appeared in the blood of 10 cases within 24 days after the feeding was commenced. Three of these infants developed a positive cutaneous reaction to almond globulin.

Analogous to the experiments cited in which protein foods were added to the regular diet but of greater practical importance are observations on normal infants which show the enteral absorption of cow's milk protein in antigenic form. In the past three years we have had opportunity to observe nine infants from one to six months of age during the period when they first received cow's milk. They were weaned entirely or were given one to two feedings of cow's milk each day to supplement maternal nursing. In all of these infants precipitin to cow's milk protein appeared in the blood from 11 to 25 days after the cow's milk was first ingested. In those who could be kept under observation for a sufficient period the precipitin was present for a time but, despite the continued ingestion of cow's milk, later disappeared (Fig. 9). These observations demonstrate that when normal infants ingest foreign protein for the first time it may be absorbed in antigenic form in sufficient amount to elicit the production of specific precipitin. After a short period the precipitin disappears, giving evidence that the absorption of antigenic protein has ceased.

The observations on normal infants indicate the presence of the same process which occurs in marasmus but in miniature and in abbreviated form. The differences, however, are striking. Gauging roughly by the degree of precipitin formation, the amount of protein absorbed by normal infants is infinitely smaller than by those affected with marasmus or diarrhœa. In the

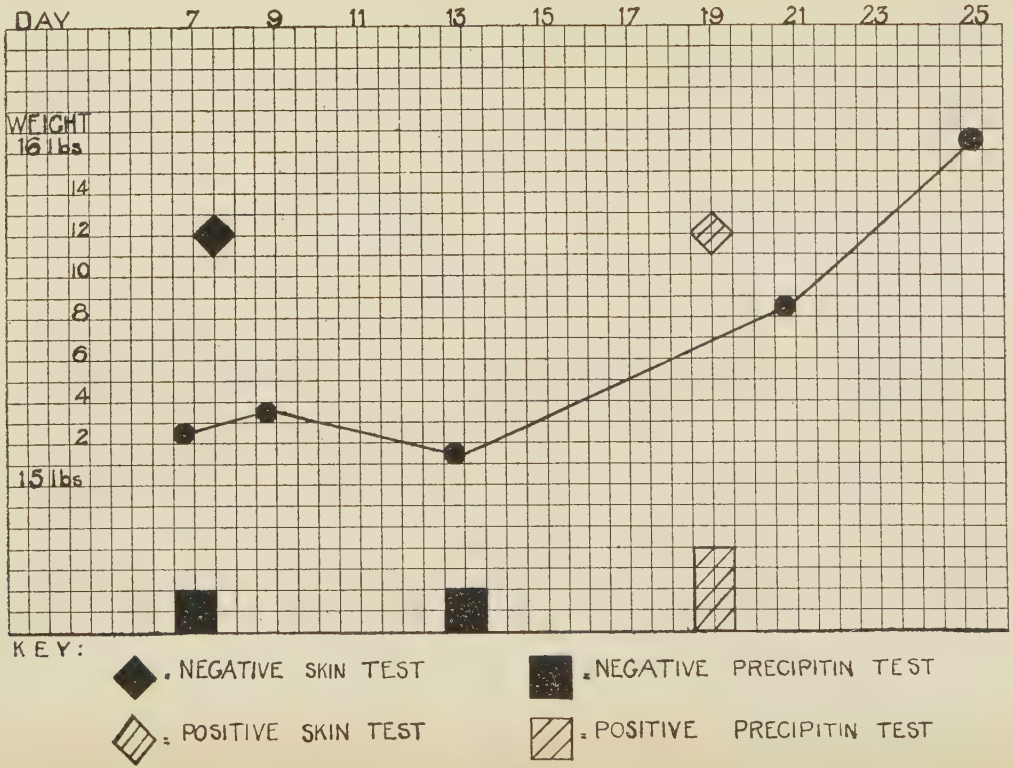


FIG. 9.—Normal breast fed infant. Illustrating the development of cutaneous hypersensitiveness to cow's milk and the appearance of precipitin for cow's milk in the blood after abrupt weaning and the ingestion of cow's milk.

normal infant an immunity reaction evidenced by precipitin formation appears very promptly, usually, from the 7th to the 15th day, while in marasmus no precipitin may be evident for many weeks. The duration of absorption of antigenic protein is also different. In normal infants precipitin is present in the blood for a very short time only and promptly disappears, showing that absorption of antigenic protein has ceased. In atrophic infants precipitin when once present is found in the blood for some time, thus demonstrating continued absorption of antigenic protein.

### V. THE FACTORS INFLUENCING THE INTESTINAL ABSORPTION OF ANTIGENIC PROTEIN

The time has now arrived when it seems necessary to consider the conditions on which the enteral absorption of antigenic protein may depend.

As pointed out by Levene and Loeb, it is upon the proteins of the organism that species distinction depends. In the mechanism of normal metabolism ingested protein derived from plants or from another animal species cannot replace directly the proteins of the human organism. It is essential that the materials for protein synthesis be served to the cells of the organism in a form which is non-specific and permits its ready organization into the proteins characteristic of the human species. This purpose is served largely by the action of the proteolytic ferments of the gastro-enteric tract in disrupting the protein molecule to its constituent amino-acids before absorption occurs.

This digestion of protein probably represents the first line of defense in preventing the possible harmful effects of the enteral absorption of antigenic protein fragments. A consideration of first importance, therefore, is whether the enteral absorption of antigenic protein by infants may be due to some interference with the process of protein digestion. In this event the intestinal wall is bathed by antigenic protein, some of which may enter the circulation.

Marriott and Davidson have suggested that the absorption

of antigenic milk protein in marasmus described by Anderson and Schloss was due probably to deficient acidity of the gastric juice. Due to this they assumed that the rennin coagulation of casein was incomplete and led to its absorption by the intestine. A brief consideration of the evidence indicates little in favor of this view. The blood of marasmic infants contains precipitin for both casein and the whey proteins which are antigenically distinct. This indicates that the whey proteins which are unaffected by rennin are absorbed in antigenic form as well as casein. Of more direct bearing are observations by Rowley who, working in collaboration with Anderson, Miss Myers and myself, found that the direct measurement of the pH of gastric juice showed no significant difference in the gastric acidity of infants who were absorbing antigenic protein from those who were not.

The earlier investigations of the proteolytic ferments in infants suffering from marasmus or diarrhœa by Reeve-Ramsey, Rosenstern, Hahn and Lust showed no significant change from normal. Recently, however, Wilbert Davidson, by means of a more accurate and dependable method, has obtained evidence that trypsin may be deficient in the duodenal contents of infants with diarrhœa. This circumstance may play a part in determining the relatively large absorption of antigenic protein which occurs in this condition. The attempt thus to explain entirely the absorption of undigested protein in all of the conditions in which it occurs leaves much to be clarified. There is no evidence at present to indicate deficient protein digestion in atrophic infants, and it seems difficult on this ground to explain the absorption of antigenic protein by normal infants. Moreover, even under normal conditions protein digestion is probably a gradual process leading to the presence of antigenic products in the intestine for a period sufficient to result in their absorption if the condition of the intestinal wall permitted. It seems probable that the permeability of the intestinal wall must be a constant influence while delayed or imperfect protein digestion may act in a quantitative way rather than as the sole determining factor.

When we reconsider the observations on infants who are normal or affected with severe malnutrition evidence is derived which seems to strengthen the hypothesis that the permeability of the intestine plays a rôle in the absorption of antigenic protein. It was noted that when normal infants were fed a protein for the first time, sufficient is absorbed to give rise to specific precipitin in the blood, but that with continued ingestion of this protein the precipitin disappears. This disappearance of precipitin indicates in all probability that antigenic protein is no longer absorbed. If at this time a new protein is fed it may be absorbed in amounts sufficient to elicit the production of precipitin specific to itself which after a time also disappears. It seems, therefore, that when a foreign protein is first ingested it is absorbed in antigenic form for a time but that with continued absorption the intestine becomes impermeable to it. It is also of importance that this impermeability seems to be specific for each protein.

Fundamentally in accordance with this view are observations made by Anderson and myself on infants with cleft palate. In our earlier observations we noted that such infants develop precipitin to cow's milk with regularity and in much larger amount than those suffering from marasmus or diarrhœa. The degree of precipitin formation by these infants compares very favorably with that of rabbits which have received intravenous injections of milk protein. It seems very probable that this great development of precipitin was dependent to a large degree on the absorption of antigenic protein by the nasal and naso-pharyngeal mucous membranes which are unprotected by digestive enzymes and are permeable to antigenic protein. Such absorption is analogous to the experience of Bresredka and of Bronfenbrenner, that guinea-pigs can be very highly sensitized by intra-tracheal injections of antigenic protein. It recalls also the observation of Blumgart that pituitary extract exerts its physiological effect when sprayed into the naso-pharynx, although it is quite inert when taken by mouth.

The course of the precipitin in the blood of these infants with cleft palate was essentially the same as in normal and marasmic

infants. After being present for a time, it disappeared completely. The precipitin disappeared despite the fact that milk protein was ingested continuously and still brought into contact with the naso-pharynx. This cessation of absorption of antigenic protein could hardly have been due to the development of digestive juices in the naso-pharynx capable of completely hydrolysing protein but probably depended on some specific change in the nasal and naso-pharyngeal mucous membranes, rendering them impermeable to foreign protein. Any attempt to explain the cause of the impermeability of the intestine under the condition cited must be largely speculative. It may be dependent on the development of highly specific enzymes or it may be the result of a definite immunologic reaction. In considering the latter possibility, it suggests that the intestinal impermeability follows the development of specific precipitin and it is not inconceivable that, at the same time, a related antibody may serve to seal the intestinal wall against antigenic protein.

The complete hydrolysis of ingested protein by the digestive enzymes was termed the first line of defense against the absorption of antigenic protein. Next to this is the protection afforded by the impermeability of the intestine to protein fragments which have not been sufficiently degraded to lose their specific character. After antigenic protein has passed into the circulation for a period a third defense mechanism becomes operative. It involves the production of specific antibodies which may divert absorbed protein from the body cells, thereby preventing its harmful effects.

It appears possible that in the physiology of protein metabolism all three defenses may be operative. Despite the efficiency of protein digestion the first ingestion of a protein may find the intestinal wall permeable to antigenic fragments which provoke an immunity reaction. Following the appearance of this reaction and perhaps related to it, complete impermeability of the intestinal wall occurs. It is due perhaps to a failure or a delay in development of these protective mechanisms that harmful effects from enteral absorption of antigenic protein may occur.

## VI. RELATION OF INTESTINAL ABSORPTION OF ANTIGENIC PROTEIN TO ANAPHYLAXIS

In conclusion, it seems necessary to discuss briefly the relation of the enteral absorption of foreign protein to the possible occurrence of human anaphylaxis. In the discussion of the harmful effects of antigenic protein absorption the terms hypersusceptibility or anaphylaxis have been avoided carefully. Assuming that such protein absorption may be harmful we have no means of demonstrating whether the harmful effects arise from direct toxic action of the protein or depend on sensitization of the body cells. If the use of the term anaphylaxis be restricted to its original meaning, designating the antithesis of immunity, no direct relationship can be definitely proved. The clinical manifestations following the enteral absorption of antigenic protein by infants bear no resemblance to the spectacular symptoms developed by the sensitized animal when reinjected with the sensitizing protein. Nor are there evident any disturbances resembling asthma, urticaria or upper respiratory irritation which belong to the group of supposed anaphylactic phenomena in the human being. The presence of an intracutaneous reaction is the only manifestation common to this group of patients and to those who absorbed antigenic protein through the intestine. But when we consider the mechanism responsible for the phenomenon of anaphylaxis rather than the actual symptoms produced, the relationship is far less remote. As pointed out by Dale, the humoral and cellular changes, on which anaphylaxis depends, indicate not an antithesis to immunity but rather a phase of its development. The typical anaphylactic experiment is an artificial condition and belongs to the experimental laboratory. It is the result of throwing a foreign protein into the circulation with a rapidity never achieved by conditions of natural absorption. It is significant that even in the highly sensitized laboratory animal no one has succeeded in inducing an enteral absorption of protein with sufficient rapidity to induce anaphylactic shock, although measures have been employed by Kleinschmidt and others which are known to induce absorption of undigested protein. When, however, we

compare the immunologic changes induced in infants by the enteral absorption of antigenic protein with those developed in animals after protein injections, we find a close similarity. Both show in their circulating blood specific precipitin and the presence of the anaphylactic antibody. This similarity is drawn closer by the fact that the anaphylactic antibody from an infant is capable of passively sensitizing a guinea-pig in the same fashion as the antibody derived from a guinea-pig previously actively sensitized. As shown by Weil, a guinea-pig treated with a foreign protein sufficient to cause the development of precipitin in its blood is very resistant to slow absorption of the protein induced by intraperitoneal injection, but it develops typical symptoms when the protein is injected into the circulation.

The defense mechanism induced in infants by the enteral absorption of protein when once established is probably adequate to render such absorption relatively harmless, or at least to mitigate its effects to a large degree. But what would happen if an infant whose blood contained anaphylactic antibody to milk were given milk by intravenous injection? The answer to this question would require the execution of a hazardous experimental venture which will never be undertaken, but one could hardly expect it to be without results.

Here the subject must rest for the present, for further discussion would extend beyond available information and could profit nothing. It seems almost indisputable, however, that the changes induced in infants by the enteral absorption of foreign protein represent an immunity reaction closely analogous to the changes induced in experimental animals when treated with parenteral injections of heterologous protein.

# THE ABORTIVE HEALING OF SYPHILIS ON THE BASIS OF EXPERIMENTAL STUDIES [1]

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THE chief aim of the chemotherapist is, as Ehrlich always pointed out, the destruction of all pathogenic organisms in an infected body. And the chemotherapist imitates the processes revealed to us by nature, in the healing of many diseases. When measles or small-pox heals, for example, the body is freed from all the pathogenic organisms residing in its tissues and contributing to it harmful influences. Ehrlich described these processes, when they resulted from the use of chemical remedies, with the coined words "sterilisatio magna." This sterilisatio may be demonstrated, at all times, by the use of various remedies in small animals harboring various protozoan diseases. We know of dyes, for example, tryparosan, trypaflavin, further arsenic and antimony preparations, one injection of which into mice or rats infected with trypanosomes or spirochætes, is followed by certain healing. Here we are dealing with an acute disease. In chronic diseases, quite naturally, the relationships are more difficult. Because of the disease, changes in the functions of organs occur in the body harboring an infection over a long period of time. This fact is generally accepted. Even before the new era of scientific chemotherapy, clinicians, on the basis of medical experience, knew that the earlier therapeutic measures, whether specific or general, were commenced, the more easily such chronic processes healed. In tuberculosis, this fact is well proved, for the earlier the disease is recognized and the earlier the therapy is commenced, the more hopeful is the healing. In the sleeping-sickness of human beings, also, it was demonstrated that when the brain was not infected the use of the empirically tried atoxyl

[1] Lecture delivered April 18, 1925.

was more effective in the early than in the later stages. Once the trypanosomes firmly established themselves in the human brain, however, atoxyl was without effect.

On the basis of his studies on mice infested with trypanosome and relapsing fever and syphilitic rabbits, Paul Ehrlich recognized salvarsan to be the most efficacious of the then known arsenobenzene derivatives. He utilized these clinical experiences and recommended salvarsan, in the first place, for the sterilization of syphilitic beings in the early stage of syphilis. Paul Ehrlich, himself, was unable to continue the experimental study of this question. Since we possess, in the rabbit, an experimental animal well-suited for the study of sterilization by means of chemical remedies, that is, of the freeing of the organism from the syphilis spirochætes, I have attacked this question from varied angles:

(1) With the aid of salvarsan, is it possible, and in what percentage, to cure biologically, that is, to sterilize rabbits experimentally infected with syphilis?

(2) Until what period of time following the infection is this sterilization possible?

(3) How does the sterilizing power of the arsenobenzene derivatives, especially salvarsan, compare with that of mercury and bismuth?

(4) In the later stages of the syphilitic infection, is it, also, possible to bring about a healing of the disease, a sterilization of the body by means of continuous treatment?

Before discussing the results of these investigations, I wish to bring before you several facts concerning re-infection, particularly superinfection in syphilitic rabbits not treated with chemical remedies.

In the following experiments, the testicle was used as the site of inoculation. When, in various time intervals following the first infection, a syphilitic rabbit was inoculated with the same strain of spirochæta pallida on the other testicle, one obtained new chancres through this superinfection regularly only until a definite period of time following the primary inoculation.

CHART 1

| Course of the first infection | Re-inoculation after days | Result and course of the re-infection |
|---|---|---|
| Typical chancre | 9 | Typical primary effect. |
| Typical chancre | 14 | Typical primary effect. |
| Typical chancre | 28 | Typical primary effect. |
| Typical chancre | 40 | Typical primary effect. |
| Typical chancre | 50 – 60 | Partly negative partly positive. |
| Chancre in involution | 60 – 70 | Usually negative, although sometimes chancres developed. |
| Chancre in involution | 70 – 90 | Almost always negative, extremely seldom a weakly developed chancre. |
| Chancre healed spontaneously or through a single treatment | 90 – 200 | Always negative. |

Reinfections still occurred occasionally from the ninth week on, but they led to the formation of typical primary effects in only a part of the animals; in the remaining animals, however, they led to infiltrations which disappeared with the involution of the primary chancre. In hundreds of animals whose chancres had healed spontaneously or through treatment with a single injection of a small, non-sterilizing dose of an arsenobenzene derivative, we never succeeded in producing a typical primary effect by reinfection after ninety days or more following the previous infection. These animals were infected usually under the skin of the testicle and also under the skin of the back with a syphilis strain used in the George Speyer House, Truffi's strain. Infiltrations with no proliferation of spirochætes appeared as the result of the second infection in only few cases. The results are in accord with the findings of A. Neisser in apes, and with those, also, of Finger and Landsteiner.

The syphilitic rabbit, therefore, behaves exactly as does the human being whose syphilis has not been healed, whose spirochætes have not been radically extirpated. During its entire

existence it never shows a re-infectio syphilitica with a typical primary effect if the same strain of spirochetes is used for re-infection. This state is characterized, as is well known, as immunity caused by infection (infectious-immunitaet). Because he still harbors the syphilis organisms in his body, the syphilitic individual, just as the syphilitic rabbit, is protected against a new infection. Should, however, the equilibrium of the immunity be disturbed, the syphilitic can, once more, become diseased through his own spirochætes; this fact is evidenced by the late manifestations (aortitis, gummas, general paresis, etc.) which appear without the acquisition of a new infection.

A new syphilitic infection with the formation of a typical chancre is, quite correctly, certain proof that the first infection was biologically thoroughly healed, *i.e.*, that the syphilitic previously sterilized was radically freed of his sprochætes. In the pre-salvarsan days, re-infection in an individual once affected with syphilis, even when long treated with mercury, was a rarity. Since the introduction of salvarsan, however, the number of syphilitic re-infections in syphilitics treated with salvarsan during the early stage has, so far as one can learn from the literature, greatly increased. Because of the nature of the disease, and the possible unreliability of the facts concerning re-infections the literature may, however, be of little worth to scientific statistics.

The question in what percentage of syphilitic individuals the treatment with salvarsan produces a sterilisatio may be exactly determined by means of animal experimentation. As is seen in the following tables, I have been able, experimentally, to answer this question. Our procedure was as follows: Rabbits, experimentally infected with human syphilis (spirochæta pallida, strain Truffi-Speyerhouse) were treated 3, 15, 20, 25, 30, 45, 60, 90, 120 days after the infection and later with three large doses of salvarsan; 110 to 120 days after the first infection or later they were re-infected with the same strain. I will present two records here as examples of the manner of conducting such investigations.

Relying upon the viewpoint of Ehrlich that the reaction of the body, which he called "Ictus immunisatorius," played a not inconsiderable rôle in all chemotherapeutical measures, I employed large doses of salvarsan, representing 2/3 of the tolerated dose. The principle followed here was to arouse this ictus as much as possible by the use of large doses, introducing, at

CHART 2

| Anzahl der Tiere | Abortivbehandlung nach: | mit: | Reinficiert nach: | Resultat + | Resultat − |
|---|---|---|---|---|---|
| | Tagen: | | Tagen: | | |
| 9 | 3 | Neosilbersalvarsan | 110 | 8 | 1 |
| 4 | 3 | Neosilbersalvarsan | 112 | 4 | 0 |
| 13 | 15 | " | 120 | 11 | 2 |
| 5 | 19 | „1495" | 100 | 5 | 0 |
| 14 | 30 | Neosilbersalvarsan | 135 | 11 | 3 |
| 9 | 45 | Neosalvarsan + Novasurol | 160 | 5 | 4 |
| 4 | 45 | Neosilbersalvarsan | 160 | 0 | 4 |
| 7 | 60 | Neosalvarsan + Novasurol | 135 | 1 | 6 |
| 6 | 60 | Neosilbersalvarsan | 135 | 1 | 5 |
| 6 | 90 | Neosalvarsan + Novasurol | 138 | 1 | 5 |
| 6 | 90 | Neosilbersalvarsan | 138 | 1 | 5 |
| 5 | 74-121 | " | 106 - 156 | 0 | 5 |
| 7 | über 170 | Silbersalvarsan | 362 - 384 | 0 | 7 |
| 4 | 172 | Novasurol | 270 | 0 | 4 |
| 4 | über 200 | Neosalvarsan | 312 | 0 | 4 |
| 2 | " 200 | „1882" | 408 | 0 | 2 |

the same time, as much of the spirochæte destroying substance (spirochætotropic) as possible. In this series of experiments, part of the experimental animals were treated with mercury and salvarsan and the other part with pure salvarsan alone. And the development of a typical primary effect after re-infection with the same strain was considered proof of the successful sterilisatio. Animals biologically healed, that is, freed from their spirochætes and their diseases, must react as do the normal toward a new infection. They must, therefore, display a long persisting primary effect of, at least, hazel-nut size which must contain innumerable spirochætes.

As seen in the chart, until the 45th day following the infection, the sterilisatio of the rabbits was successful in a great percentage of cases, despite the giving of only three injections of large salvarsan doses (approximately 2/3 dosis tolerata). After the 45th day, however, the relationships were reversed, the sterilization was only seldom successful. The re-infections of most animals were without result and a primary effect practically rarely developed. For these series, treated from the 90th day on, however, I never succeeded in re-infecting a previously treated animal; I never succeeded in producing a primary effect. And the opinion of P. Ehrlich, accepted today by many clinicians, that the importance of salvarsan lies in the early treatment of syphilis is now experimentally proved to be correct. From these investigations we must conclude that the fate of the syphilitic rabbits, in regard to healing, is decided in the first four to six weeks following the infection. And, as indicated by many facts, the same statement pertains to human beings. To assign this period of 45 days, obtained in rabbits, to human beings without qualifications, would be, naturally, quite false. The space of time, in which, in human beings, the sterilization is successful in a high percentage of cases is perhaps even larger. It was proved experimentally, further, that when compared with the results following the use of salvarsan alone a greater percentage of cures did not follow the combined treatment with mercury and salvarsan. This fact stands in harmony with the already mentioned experiences of physicians, that by treatment with mercury alone, biological healing of syphilis in human beings is seldom attained. And it is in accordance, also, with the fact that salvarsan without mercury in the early treatment of syphilis affords results equally as good and as permanent as those obtained by the combined salvarsan and mercury therapy.

The inability to sterilize a part of the syphilitic animals, even when salvarsan or the combined therapy was commenced a few days following the infection, is of special interest (Chart 1). In a group of 26 animals treated during the first days of the infection, three did not respond to re-infection, that is they

remained syphilitic; one of these was treated with combined therapy three days following the infection.

Since large doses of the salvarsan preparations were used, since the spirochætes were unable to multiply effectively during this short period of time (three days), and since, further, the sterilization was effective in the same percentage of cases even after 30 days, the failure must be sought, not in insufficient dosage, but in the heterogenous reactions of the infected animals to the salvarsan introduced. Whether one attributes

CHART 3

Abortivbehandlung nach 15 Tagen.

| Kan.-Nº | Inficiert am: | Behandelt am: | Behandlungsmodus | Reinficiert am: | Befund | |
|---|---|---|---|---|---|---|
| 1409 | 31.I.21 | 15.VI.21 | 0,11 Neosilbersalvarsan + 0,005 Novasurol p. kg | 21.IX.21 | Sp++g. Infr. gute Schanker, 0 Erscheinung. | |
| 1411 | " | " | " " " " | " | | |
| 1412 | " | " | " " " " | " | Sp+++g. Infr. g. Schanker | |
| 1413 | " | " | " " " " | " | Sp++ + g. Infr. g. Schanker | |
| 1414 | " | " | " " " " | " | Sp+++g. Infr. g. Schanker | |
| 1416 | " | " | " " " " | " | Sp + g. Infr. g. Schanker | |
| | | 2. Injektion 25.VI.21 | 0,05 Neosilbersalvarsan + 0,004 Novasurol p. kg | | | |
| 1438 | 10.VI.21 | 25.VI.21 | 0,11 p. kg Neosilbersalvarsan | 30.II.21 | Sp ++ g. Infr. Schanker | † |
| 1439 | " | " | " " | " | Sp ++ g. Infr. g. Schanker | |
| 1440 | " | " | " " | " | Sp + 2 Infr. g. Infr. | |
| 1441 | " | " | " " | " | Sp + g. Infr. g. Schanker | |
| 1442 | " | " | " " | " | Sp + 2 Infr. g. Schanker | |
| 1443 | " | " | " " | " | 0 Erscheinung | |
| | | 2. Injektion 5.VII.21 | 0,05 p. kg Neosilbersalvarsan | | | |

this result to a deficient Ictus immunisatorius or to the incapability of the body to react is unessential for the facts. From these experiments we find that even the greatest doses do not produce healing in 100 per cent. of syphilitics treated in the early stage in contrast to the results obtained in relapsing fever or trypanosome diseases in mice. Among the factors deciding the fate of the syphilitic in regard to healing during the first months following the infection not only the spread of the infection but also the ability of the body to react and the Ictus immunisatorius certainly play a great rôle. Since we do not know the obvious individual variations, the course of the infection, the

processes of healing and the constitutional variations, the possibility of healing, even in the early treatment, cannot be foretold.

In the experimental plan used here to prove the sterilization of syphilitic animals, a method is given by means of which the efficacy of other antisyphilitic remedies in human therapy may be experimentally tested and compared to that of salvarsan. I wish to inform you of the results obtained in the testing of mercury and bismuth by the use of this method.

Paul Ehrlich, with the aid of animal experimentation, discovered salvarsan and drew conclusions of clinical value as to the importance of this medicament in human therapy. In the same manner, the worth of a remedy as compared with salvarsan may be determined.

Based upon the usefulness of large doses of salvarsan in the early stage of syphilis, as was advocated by Ehrlich, and upon its substantiation through animal experimentation the following dosage comes into consideration:

| | |
|---|---|
| Neosilversalvarsan ..... | 0.45 gms. |
| Neosalvarsan ..... | 0.5–0.6 gms. |
| Silversalvarsan ..... | 0.3 gms. |

These correspond, approximately, to 2/3 of the dosage tolerated by human beings (dosis tolerata).

The quoted investigations confirm the significance, in the early stage of syphilis, of the pure salvarsan therapy, with the use of large doses; a therapy used by many clinicians. When these findings, obtained principally from animal investigation, are applied to human therapy, then three, perhaps four and at most five large salvarsan doses should suffice to produce biological healing in the early stage, which is the only time at which complete success may be expected. Whether a greater percentage of cures are attainable through the use of more injections appears doubtful to me on the basis of my investigations. A sterilization in the biological sense, that is, a healing, a complete freeing of the body from the spirochætal infection may also be produced in the early stage by the use of small doses of salvarsan.

Through these experiments it was proved that the injection of three large doses of salvarsan, which leads to abortive healing

in the early stage, that is, up to approximately 4-6 weeks following the infection, does not effect a sterilization of the infected body in the later periods. And now there remained to be investigated the possibility of attaining a healing of the disease in the later stages of the infection by means of a long-continued treatment. The long-continued intermittent treatment is certainly a common practice in medical therapeutics of human beings. Physicians have made the observation, however, that in the later stages of syphilis the Wassermann reaction in some individuals, irrespective of the therapeutic measures used, cannot be made to disappear. It is also known that many syphilitics, despite intensive treatment over many years, become sick and die from the late stages of syphilis. I need only mention here the general paresis, tabes, aortitis syphilitica and gummas of the internal organs and of the skin. These diseases occur despite continuous treatment with all possible remedies.

The following examinations represent not only a control of previous experimental results but also supplement and control the clinical experience.

The intermittent continued treatment used in the attempts to sterilize syphilitic rabbits in the later stages of the infection, that is 4 to 12 months following the inoculation, was modeled after the therapy used in those syphilitic individuals not healed during the early stage by the abortive treatment. A part of the animals were treated systematically with a large number of individual injections of the arsenobenzene derivatives, another part with arsenobenzene derivatives plus mercury preparations, for example, hydrargyrum salicylicum. A considerable number of the animals died intercurrently, and some died, due, certainly, to the continuous administration of the chemotherapeutic agent. I will present the record of an experiment to demonstrate such a systematic treatment. Dr. Evers and Miss Moebus, the laboratory assistant, participated in the carrying out of this and the remaining experiments.

As seen in the summary (Chart 4) the treatment, in all animals was composed of 3–4 (6 to 9 months) courses, each comprising 16–20 injections. Six weeks after the completion of the

course the animals were re-infected. And in none of the 14 animals treated in such a continuous manner was a re-infection successful. This finding appears to me of fundamental theoretical and practical significance. Until other attempts to explain this fact on an experimental basis are possible, one must say, that in the once syphilitic animal, despite continued treatment, the syphilitic infection remains latent. Opposing the hypothesis that following the healing of the infection, the rabbits harbor

CHART 4

Dauerbehandlung mit Kuren.

| Kan. Nr. | inf. am | Beginn der Kur | Präparate | Dauer der Kuren | reinf. am | Resultat |
|---|---|---|---|---|---|---|
| 1747 | 20. XII. 1921 | 2. III. 1922 | 3 × 0,08 Neosilbersalv. iv. | 7 Mon. | 11. XII. 1922 | — |
| 1750 | 20. XII. 1921 | 2. III. 1922 | 7 × 0,06 Neosilbersalv. iv. | 8 „ | 11. XII. 1922 | — |
| 1753 | 23. XII. 1921 | 3. III. 1922 | 1 × 0,05 Quecksilberpräp. | 7 „ | 11. XII. 1922 | — |
| 1794 | 3. I. 1921 | 3. III. 1922 | 9 × 0,03 276 intram. | 7 „ | 11. XII. 1922 | — |
| 1762 | 23. XII. 1921 | 4. III. 1922 | 2 × 0,08 / 8 × 0,06 Neosilbersalv. iv. | 7 „ | 11. XII. 1922 | — |
| 1763 | 23. XII. 1921 | 4. III. 1922 | 1 × 0,05 Quecksilberpräp. | 7 „ | 11. XII. 1922 | — |
| 1724 | 8. XII. 1921 | 10. III. 1922 | 1 × 0,04 / 8 × 0,03 276 intram. | 7 „ | 11. XII. 1922 | — |
| 1756 | 23. XII. 1921 | 10. III. 1922 | 3 × 0,08 / 7 × 0,06 Neosilbersalv. iv. | 7 „ | 11. XII. 1922 | — |
| 1686 | 10. XI. 1921 | 3. V. 1922 | | 8 „ | 11. XII. 1922 | — |
| 1733 | 24. XII. 1921 | 3. V. 1922 | 10 × 0,03 Quecksilberpräp. | 8 „ | 11. XII. 1922 | — |
| 1738 | 20. XII. 1921 | 3. V. 1922 | 276 intram. | 8 „ | 11. XII. 1922 | — |
| 1906 | 12. IV. 1922 | 15. VIII. 1922 | 1 × 3 ccm (0,3) 1882 iv. | 8 „ | 16. V. 1922 | — |
| 1908 | 12. IV. 1922 | 15. VIII. 1922 | 1 × 4 „ (0,4) / 1 × 8 „ (0,8) 2203 iv. | 8 „ | 16. V. 1922 | — |
| 1876 | 11. III. 1922 | 8. VIII. 1922 | 1 × 4 „ (0,2) — 2158 / 1 × 4 „ (0,4) — 1882 / 8 × 8 „ (0,4) — 2203 iv. | 8 „ | 16. V. 1922 | — |

the syphilitic virus no longer and become therefore, immune, are important experimental proofs and generally recognized experiences in the syphilis of human beings and rabbits. And positive inoculation attempts effected by means of the blood and organs of latent-syphilitic individuals and animals, prove that the insusceptibility of the syphilitic individuals is due to a latent infection, therefore, a so-called "Infectious immunitæt"—immunity caused by infection. The syphilitic free from all symptoms for years is taken ill with late manifestations containing spirochætes, *i.e.*, gummas, aortitis, general paresis, tabes, dorsalis, etc. Rabbits, once infected with syphilis, after being symptom-free for several months acquire in 20–25 per cent. of the cases

keratitis, a local recurrence, or gumma-like papules. These once syphilitic individuals are, also, during the latent period, insusceptible to a new infection with the formation of a typical primary effect. As was previously mentioned, I have, experimentally, in a very large material, confirmed this fact by re-infections in rabbits. The positive inoculation with organs of such animals confirms the assumption that the immunity to new infections is actually both in syphilitic rabbits and in syphilitic individuals, only a specious immunity, a resistance due to the latent infection, preventing a new infection starting the development, on the site of inoculation, of the manifestations of the disease with the presence of spirochætes. This statement is not contradicted by the experimental experience of Landsteiner, who found that no small percentage of chronic syphilitic men react to re-infection in the later stages of the infection, with local findings which correspond to the stage of the infection.

In the abortively healed animals in my previously discussed groups of investigations, the origin of a typical primary effect following re-infection is to be considered definite evidence that a resistance or immunity due to the harboring of spirochætes in the body is not present.

The question why after a definite interval of time following the first infection, and despite the use of large doses of the arsenobenzene derivatives, the sterilization of syphilitic animals does not succeed, has a theoretical significance. The explanation that after a definite period of time, the syphilitic spirochætes become cell parasites and are, therefore, unaffected by salvarsan, appears hardly plausible. It is improbable that the spirochætes are found, at first, only in the lymph and tissue spaces on the site of inoculation. For already in the early stage of the syphilis infection, a part of the penetrated spirochætes are demonstrable in cells whether they be in the site of infection filled with leucocytes, in the cells of the lymph-glands, in the bone marrow, in the spleen; they are, therefore, intracellularly deposited.

All experimental and clinical experiences disprove the assumption that the spirochætes lead only from a definite time on, to a generalized infection. The attempt to destroy the site of infec-

tion and its surrounding tissue by excision or cautery once a syphilitic infection has occurred, shows that very early, perhaps only a few hours following the infection, the spirochætes are carried into not only the tissue surrounding the primary effect, but also into the glands and from there into the lymph, blood, and internal organs. When one considers the well-known Schimmelbusch experiments with the much larger and non-motile anthrax bacillus, this result is to be expected.

With regard to our present knowledge and conceptions of the nature of the syphilitic infection, the ictus chemotherapeuticus of Paul Ehrlich and the so-called " Umstimmungs theory " of Jadasohn most adequately explain why a sterilization in the once syphilitic body occurs almost always only in the first four weeks following the infection. Since during the first 30 days, 80 per cent. of the animals can be healed abortively by the use of only a few doses of salvarsan, and in from 60 to 90 days only 10 per cent. respond despite many injections, some factor which influences the action of the salvarsan preparations must have entered during the course of the infection. An alteration in the syphilitic body resulting from the chronic infection, and displaying an altered capability of reaction occurs after a definite period of time. The syphilitic body infected a long period of time, therefore, reacts differently to the injection of the salvarsan preparations. In the freshly infected animal the arsenobenzol derivatives call forth a reaction describable as ictus chemotherapeuticus; this reaction is, obviously, necessary for healing and for the total destruction of all spirochætes in the body. Due to the altered tissue, reaction in the chronic infected body, this ictus chemotherapeuticus, so necessary for sterilization, is not called forth and all spirochætes are not, with certainty, destroyed. The nature of this alteration is not as yet clarified. Just as the conception of tissue alteration is very useful for the explanation of the peculiarities of the late syphilitic symptoms, for example, the rapidly proliferating, richly cellular but spirochæte poor gummas, so, it appears to me, is the alteration in the organism utilizable in explaining the unsuccessful or seldom successful

sterilization in the late stages of rabbit syphilis by the very same remedies so efficacious in the early stage.

It was very interesting to compare the sterilizing effect of the mercury preparations with the results obtained by the treatment with the arsenobenzene derivatives, especially salvarsan. Mercury, the oldest anti-syphilitic, was the remedy of choice until the discovery of salvarsan. On the basis of clinical experience, it should be accepted that a sufficiently intensive treatment with mercury over a sufficient length of time leads to a sterilization of the infected body in many syphilitic human beings. It must remain clear in the experimental testing of this question, however, that mercury acts differently from the arsenobenzene derivatives and this pertains also to bismuth. I have studied this question for years and, on the basis of these studies, I wish to state that neither mercury nor bismuth has a direct annihilating action in the sense experimentally demonstrated with the arsenobenzene derivatives. The bases for this assertion are the following:

(1) Mercury and bismuth, by every manner of administration, act much more slowly than the arsenobenezene derivatives. For example, following the intravenous injection of silver-salvarsan, the spirochætes, often after only a few hours, disappear permanently from the syphilitic individual, never to reappear. After bismuth and mercury therapy, however, the spirochætes are destroyed only very slowly during the course of many days.

(2) Bismuth and mercury preparations are essentially active only when deposited in the body in depot form. The effect of bismuth, therefore, depends upon arresting development, by means of which, under certain circumstances, a sterilization may be attained.

(3) Using intravenous administration, the effective dosage of mercury and bismuth compounds almost or actually equals the tolerated. And the preparations exhibit a very small therapeutic index. These findings, also, indicate the absence of a direct effect by these preparations on the parasites. In their use the ictus chemotherapeuticus which leads to sterilization and which characterizes, essentially, the attack on and the annihilation of the parasites by the chemotherapeutica is absent.

In the experimental testing of this question, I used mercury preparations, demonstrated by human therapy to be the most effective. The treatment consisted of intramuscular injections of mercury preparations and was commenced on the fifteenth day following the infection. The rabbit is very sensitive to mercury. Yet, by using quantities definitely smaller than the lethal dose, it was possible to give a number of injections without a great loss in animals. Preceding their use, the toxic dose of all preparations was determined, and this included the acute fatal dose following intravenous administration, the chronic fatal dose following intramuscular injection, and the accurate tolerated dose. Two-thirds to three-fourths of the safely tolerated dose was employed in this treatment. And the animals were re-infected, as in the previous investigations, 4½ months following the first infection. Since these experiments were conducted with the same strain used in the same investigations with arsenobenzene derivatives, they are eligible for comparison with the previous series of investigations.

As seen in the charts displayed, in only a small percentage of the syphilitic rabbits, as compared with the percentage healed by means of salvarsan, did the use of *mercury* produce a cure. In regard to the therapeutic effect, striking differences were shown in the individual series of experiments. These variations depend, obviously, upon differences in absorption and, correspondingly, in the therapeutic action. Since mercury compounds, in comparison with salvarsan, act slowly and do not directly destroy the spirochætes, since, further, the course of the infection varies, may progress more rapidly or more slowly, in individual series or animals, with differences in the absorption of mercury depots the curative effects of these slowly acting preparations is irregular. With the use of intravenously administered salvarsan, this is not the case. The rapidity of absorption of the mercury preparations, on the one side, and the status of the infection, on the other, therefore, influence the results. Through the arrestment of spirochætal development and the prevention of definite manifestations, healing is simulated; the question of the curative action of the mercury depots, therefore, appears to

us now in an other light. The process of re-infection has served for its clarification. While in the abortive treatment with salvarsan, curative results are obtained in 80–90 per cent. of the syphilitic rabbits, with the use of mercury, under the same conditions, only some 37 per cent. are healed.

The investigations summarized in the Chart 5 served as a control for the series of experiments. Healthy rabbits were pro-

CHART 5

Abortivbehandlung mit Hg-Präparaten.

| Anzahl d Tiere: | Abortivbehandlung nach Tagen: | Abortivbehandlung mit: | Reinfiziert nach Tagen: | Resultat + | Resultat − |
|---|---|---|---|---|---|
| 3 | 3 | ChA 276 | 135 | 2 | 1 |
| 3 | 14 | " " | 135 | 3 | 0 |
| 3 | 15 | Hg salicyl. | 135 | 0 | 3 |
| 5 | 15 | Calomel | 135 | 3 | 2 |
| 4 | 15 | Anthranilsaures Hg | 135 | 3 | 1 |
| 6 | 15 | ChA 382 | 135 | 0 | 6 |
| 5 | 14 | ChA 276 | 135 | 2 | 3 |
| 6 | 15 | Hg. salicyl | 135 | 0 | 3+3 Rec |
| 1 | 15 | ChA 337 [Bi+Hg] | 135 | 0 | 1 |
| 1 | 15 | ChA 315 [Bi+Hg] | 134 | 1 | 0 |

phylactically treated with mercury and then infected. And in all animals the infection took place with the development of a primary effect. The prophylactic treatment with mercury preparations had, therefore, no protective effect. This proves that the negative result of the re-infections, as seen in the chart, depends upon a latent infection.

I have now come to the consideration of the therapeutic efficacy of *bismuth*, in its relation, especially to the abortive healing of experimental syphilis in rabbits. These investigations, analogous to the just discussed experiments with mercury depots,

were conducted in the expectation that through arresting spirochætal development the continuous action of the bismuth would lead to a slow sterilization of the syphilitic rabbits. But with these experiments great difficulties arose and were revealed by the control investigations. A great number of normal non-infected animals were treated in the same manner as the syphilitic rabbits, with the formation of bismuth depots. And in both series the re-infection, respectively the infection with syphilitic material, was undertaken. A surprising result was obtained; the normal animals harboring bismuth depots did not react to the infection with the formation of a chancre. I do not wish to

CHART 6

Vorbehandlung mit Hg-Präparaten.

| Zahl der Tiere | Präparat | infiziert nach Tagen | Resultat + | |
|---|---|---|---|---|
| 4 (2†) | 1×0,01 Novasurol iv. | 21 | 4 | 0 |
| 2 (3†) | 3×0,03 p. kg 337 intram. | 135 | 2 | 0 |
| 1 (4†) | 3×0,01 Hg salicyl. intram. | 135 | 1 | 0 |
| 2 (3†) | 3×0,009 p. kg Kalomel intram. | 135 | 2 | 0 |
| 3 (2†) | 3×0,01 p. kg Hg salicyl. intram. | 135 | 3 | 0 |

infer here that the animals did not become infected; they merely did not react with the formation of a syphilitic primary effect characteristic of the freshly acquired syphilitic infection in almost 100 per cent. of rabbits as in previously syphilis-free human beings.

On the basis of these surprising findings, I have attacked, in the first place, the study of the *prophylactic* action of the bismuth depots. The unexpected finding was thereby obtained that, in contrast to the arsenobenzol derivatives and the mercuric preparations which afforded only a short-lasting protection to the syphilitic infection, the bismuth depots, for a period of six months or more, prevented the acquiring of an infection with the building of a primary effect. As seen in the following chart, various bismuth preparations, water soluble and also completely water insoluble preparations, as, for example, bismuth carbonate, were examined.

In 68 previously untreated animal controls, inoculated at the

same time and with the same material, the infection took root in 94 per cent. of the cases, with the formation of a syphilitic primary effect. Serving also as controls were animals injected with easily absorbable and diffusible, non-depot-forming bismuth preparations. These are the 9 animals, shown on the chart, which were treated with preparations 414 and 470. As the roentgen examination showed, the greatest part of these preparations was absorbed from the muscles in approximately 8 days; after 20 days the absorption was absolutely complete. If the

CHART 7

Schutzwirkung der Wismut-Depots, gegenüber der Syphilisinfektion.

| Anzahl d. Kan. | Vorbehandelt mit | Infiziert nach Tg | Resultat + | Resultat − | Röntgenbefund | | Kontrolle + | Kontrolle − |
|---|---|---|---|---|---|---|---|---|
| 2 | Nr. 286.3 × je 0,1 | 55 | 0 | 2 | starkes Depot | | 8 | 0 |
| 2 | Nr. 464.1 × 0,3 | 11 | 0 | 2 | starkes Depot | Organverimpfg. | 7 | 0 |
| 6 | Nr. 464.2 × 0,2 | 106 | 0 | 6 | starkes Depot | Verimpfg. d. Eiters | 5 | 0 |
| 3 | Bism. carb. 0,3 | 11 | 0 | 3 | starkes Depot | | 7 | 0 |
| 2 | „ 0,3 u. 0,2 | 53 | 0 | 2 | starkes Depot | | 8 | 0 |
| 3 | „ 0,3 | 61 | 0 | 3 | starkes Depot | Verimpfung | 7 | 1? |
| 1 | „ 0,3 | 67 | 0 | 1 | | | 6 | 1 |
| 3 | „ 0,2 | 69 | 0 | 3 | starkes Depot | Verimpfung | 15 | 2 |
| 3 | „ 0,2 u. 0,3 | 89 | 0 | 3 | starkes Depot | | 5 | 0 |
| 1 | „ 0,2 u. 0,3 | 109 | 0 | 1 | starkes Depot | | | |
| | Kontrollen mit nicht Depotbildenden Bi-Verbindungen. | | | | | | | |
| 5 | 470 { 2 × 0,08 / 1 × 0,06 | 68 | 5 | 0 | | | 8 | 0 |
| 4 | 414,2 × 0,05 | 14 | 3 | 1 zweifelhaft | Schattenspur | | 3 | 1 |

assumption that the bismuth depots prevent the development of a primary effect, that is to say condition the non-acquisition of the infection, be correct, then in those animals in which the same amount of bismuth was introduced intramuscularly, but in which the depot was absorbed, the infection should take root. And this was actually the case. The development of the chancre was doubtful in one animal, similar to one of the control animals of this series. It was, thereby, proved, so far as the occurrence of an infection may be inferred from the appearance of a chancre that the bismuth depots, namely, the bismuth deposit lying in the tissues in plug form, prevented the acquisition of a syphilitic infection with certainty until the fourth month.

The question now remained to be clarified whether the non-development of a primary effect in those animals containing bismuth depots actually denoted the absence of an infection. To this purpose, animals containing bismuth depots were infected, the depots were retained and after two to three months they were removed. A depot formed for example, in the ear, was later removed by amputation of the ear; a bismuth depot formed in the thigh was excised. Surprising results were obtained. In such animals, four to five months after the primary infection, chancres developed on the sites of inoculation; the syphilitic spirochætes had remained dormant four to five months in the area of infection. They were unable to multiply, because the bismuth in smallest quantities was circulating in the body fluids, but they were not dead. And the bismuth depots had produced only a state of equilibrium between the pathogenic organisms and the infected body, as it is found in the latent stage of the syphilitic infection. It was experimentally proved here, for the first time, that under the development-arresting influence of a chemical substance—in the present case, a bis-

CHART 8

Kaninchen № 3102.

| | |
|---|---|
| 8. VIII. 24. | 2050 gr. 0,2 g. Kg Ch. N. 332 4/Tel subc. = 1,025 cc ½ in jede Ohrmuschel. |
| 13. IX. 24. | 1900 gr. inf. an beiden Hoden von K. 3120 Kontr. 3155-60. |
| 1. X. 24. | bds. kaum infiltriert, Depots fast leer. |
| 13. X. 24. | unverändert. |
| 23. X. 24. | bds. fast glatt. |
| 3. XI. 24. | bds. fast glatt. |
| 13. XI. 24. | bds. fast glatt. |
| 20. XI. 24. | bds. glatt. |
| 28. XI. 24. | Coüpieren beider Ohren unterhalb d. Depots. 2150 g |
| 21. I. 25. | R. bohnengroß flach m/Schorf, L. glatt. |
| 26. I. 25. | R. bohnengr., flach m/stark. Schorf. |
| 5. II. 25. | R. wallnußgroß, flach. |
| 18. II. 25. | R. wallnußgr., flach, gut absorb. Verhältnisse. |
| 3. III. 25. | R. wallnußgr., bezinnender Hodenvorfall. |

Alle 6 Kontrollen sind angegangen wie folg. Beispiel:

Kaninchen № 3155.

| | |
|---|---|
| 13. II. 24. | inf. an beiden Hoden von K. 3120 |
| 30. II. 24. | bds. Knoten. |
| 13. I. 24. | bds. erbsengroß, R wenig vereitert. |
| 23. I. 24. | bds. gut bohnengroß. |
| 27. I. 24. | R. Spir. ++ g. bew.; L. Spir. +++ g. bew. |

muth preparation—the spirochætes remain latent on the site of infection, that, so to speak, a "slumbering" of the infection may occur. In the completely normal tissue of the site of inoculation, from which all inoculated particles are absorbed and on which no form of infiltration is palpable, the spirochætes lie alive, perhaps in a resting form whose morphology we do not as yet know.

The attempts to study the sterilizing power of the bismuth depots were carried out after the same procedure. Syphiliticly infected animals retained their depots and these were extirpated after three, four and five months. When the ear was used it was amputated; when the thigh was used, the depot was dissected - out.

CHART 9

Kaninchen 3111.

8. VIII. 24. 2300gr. 0,2 pKg ChA 332 i/Cel subc. =
= 1,15 cc $\frac{1}{5}$ in jede Ohrmuschel.

13. IX. 24. inf. an beiden Hoden v. 7.3120 2250gr
Kontr. 3155-60.

1. X. 24. bds. infiltriert; Depots bds. Kl. mit retrol. Belag.
13. X. 24. R- Knötchen. L- kaum infiltriert.
23. X. 24. R- Kl. Infiltrat. L- glatt.
3. XI. 24. R- fast glatt; L- glatt.
13. XI. 24. bds. glatt.
20. XI. 24. " bds. glatt.

28. XI. 24. Cauterieren beider Ohren unterhalb der Depots.

21. I. 25. R- wallnußgr. L- glatt 2250gr
26. I. 25. R- pflaumengr.
27. I. 25. R- Spir. +++ g. bew.
4. II. 25. R- Spir. + g. bew.; vereitert.

This series of experiments is not, as yet, completed. But from what I have shown you here it is unlikely that with the use of bismuth depots we will obtain the sterilization of an infected body in as high a percentage of cases as is obtained by the aid of the soluble arsenobenzene derivatives or of mercury.

Whether by the use of bismuth preparations a real sterilization may be attained, or whether these depots produce solely a continuous latent state, as is seen, elsewhere, during the course of syphilis following an unsuccessful primary abortive healing, is material for further experimental examination and also for clinical experience. For often, after two, five and ten years, in the therapeutically treated or untreated syphilitic devoid of

syphilitic manifestations, the late forms aortitis, gummas, etc., may suddenly appear.

Summarizing, I wish to specify the results of the investigations I have discussed before you here under the consideration of the great and valuable experiences of the clinicians with the arsenobenzene derivatives, mercury and bismuth compounds:

(1) The specific therapy in a syphilitic individual should be commenced as soon as possible.

(2) The sterilization of the infected body is successful in a high percentage of cases until a definite time interval. Animal experimentation and the experience of clinicians seem to agree that the time interval in which an abortive healing with salvarsan is possible amounts to approximately four to six weeks after the date of infection, in human beings it may be, however, stretched to two months.

(3) After this period of time, as animal experimentation and clinical experience agree, a sterilization of the syphilitic body, even through continued intermittent treatment, is usually not attainable.

(4) Mercury, in the form of muscle depots, can lead to the curing of experimental syphilis in rabbits but in a much smaller percentage than obtained by the use of salvarsan.

(5) The question, whether bismuth alone can produce a sterilization is not, as yet, conclusively decided, neither by experimental methods nor through clinical experience.

(6) Both mercury and bismuth are, however, because of their development-arresting and their general antisyphilitic action, valuable as supportive remedies to the direct spirochæte-destroying salvarsan and should be incorporated in the therapy of the early and late stages of syphilis.

(7) Since, on the one hand, these preparations do not directly destroy the spirochæte and, on the other, exert chiefly a continuous effect resulting from a slow absorption and since, further, when introduced into the vascular system, they exert a toxic action, and show only a chemotherapeutic index, the intravenous injection, whether alone or combined with a salvarsan preparation, is, therefore, not recommendable; the intramuscular use comes much more in question.